# Electrochemical Glucose Sensors and Their Applications in Diabetes Management

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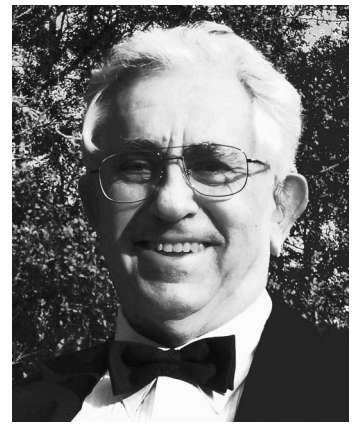
# 1. Scope

## 1.1. Coverage

About 6,000 peer reviewed articles have been published on electrochemical glucose assays and sensors, of which 700 were published in the 2005–2006 two-year period. Their number makes a full review of the literature, or even of the most recent advances, impossible. Nevertheless, this review should acquaint the reader with the fundamentals of the electrochemistry of glucose and provide a perspective of the evolution of the electrochemical glucose assays and monitors helping diabetic people, who constitute about 5% of the world's population. Because of the large number of diabetic people, no assay is performed more frequently than that of glucose. Most of these assays are electrochemical. The reader interested also in nonelectrochemical assays used in, or proposed for, the management of diabetes is referred to a 2007 review of Kondepati and Heise.[1]



Adam Heller was born in 1933. Surviving the Holocaust, he arrived in Israel in 1945. He received his M.Sc. in Chemistry and Physics in 1957, then his PhD in Organic Chemistry in 1961 from Ernst David Bergman at the Hebrew University in Jerusalem. He postdoced at UC Berkeley (1962-3) and at Bell Laboratories (1963-4). At GTE Labs (1964–1975) he built the first $Nd^{3+}$ liquid laser and, with J. J. Auborn, the still worldwide used Li/$SOCl_2$ battery. At Bell Labs (1975–1988) he designed the first >10% efficient electrochemical solar cells and the first >10% efficient hydrogen-generating solar-powered photoelectrode. He also headed Bell Labs' Electronic Materials Research Department (1977–1988), which developed part of the high density chip interconnection technology underlying the miniaturization of portable electronic devices. He was appointed to the Ernest Cockrell Sr. Chair in Engineering of the University of Texas at Austin in 1988, and in 2001 became one of UT's first Research Professors. At UT he pioneered the electrical wiring of enzymes. In 1996 he cofounded with his son Ephraim Heller TheraSense Inc., now part of Abbott Diabetes Care, to improve the lives of diabetic people. The company introduced in 2000 the blood sugar monitor *FreeStyle*™, a thin-layer microcoulometer utilizing only 300 nL of blood, so little that it was, for the first time, painlessly obtained. In 2007 it provided for more than 1 billion painless glucose assays. After alleviating the pain of diabetes monitoring, *FreeStyle Navigator*™, based on the electrical wiring of glucose oxidase, introduced in 2007 in Europe and Israel and in 2008 in the U.S., removed the worry of diabetic people by continuously monitoring their glucose levels. Heller aims his work at alleviating suffering through bioelectrochemistry.

## 1.2. Exclusion of Studies on Glucose Electrooxidizing Anodes of Cardiac Assist Devices, Pacemakers, Waste-Utilizing Electrical Power Generators, and Bioelectronic Devices

Historically, glucose electrooxidizing anodes have been studied not only because of their importance in diabetes management, but also in the context of glucose-$O_2$ biofuel cells. The objectives of biofuel cell research were generally overambitious. After 40 years of research, there is not a single biofuel cell in use. Originally, the biofuel cells were intended to power cardiac assist devices ("artificial hearts"),[2,3] then cardiac pacemakers,[4–6] then to supply electrical power to homes or electrical grids by electrooxidizing glucose in, or derived of, wastes.[7–9] The earliest studies already identified insurmountable power density and stability associated limitations, but these were not recognized by all investigators. Today, the power density of the glucose-$O_2$ biofuel cells remains about $10^4$ fold below that required for a cardiac assist device and about $10^3$ fold below that necessary to competitively supply power to the electrical grid. Furthermore, the operational lives of low-power-density biofuel cells for cardiac pacemakers are about $10^3$ times shorter than required. Recent research, undertaken in the context of bioelectronic devices, has no defined application—the bioelectronic devices in which they are to be used have not been specified.[10–16] Hence, this review covers only the glucose anodes of those disposable biofuel cells that might provide for

Ben Feldman received his Ph. D. from the University of North Carolina/Chapel Hill in 1986, for electrochemical studies of electron transport through polymeric and crystalline thin films, under the direction of Dr. Royce Murray. This was followed by postdoctoral stints at the IBM Almaden Research Center (quartz crystal microbalance electrochemistry) and the USDA Albany Research Center (electrochemistry of Nitrogenase FeMoco). In 1990, he joined the faculty of UCSF to specialize in electrochemical determination of low level Pb in blood. In 1995, he joined TheraSense, Inc., where he lead development of the first commercially available submicroliter blood glucose test strip, as well as a redox polymer-based continuous glucose sensor. He is currently Director of Advanced Development at Abbott Diabetes Care in Alameda, CA.

a few weeks the low power required by subcutaneously implanted glucose sensors.

## 2. Roots and Fundamentals

### 2.1. Direct, Nonenzymatic, Electrooxidation and Electroreduction of Glucose

Glucose was directly electrooxidized to gluconic acid in a sulfuric acid solution at a lead anode in 1909 by Walther Loeb.[17] In 1937, the Atlas Powder Company manufactured sorbitol commercially by electroreducing glucose in a NaOH-$Na_2SO_4$ solution at an amalgamated lead electrode in a diaphragm cell.[18] Studies of direct electrooxidation[19] and electroreduction[20] of glucose in basic (pH > 11) and acidic (pH < 1) solutions continue to date. At pH 7.4 glucose has been directly electrooxidized, at a current density of 1 mA $cm^{-2}$, on an electrode coated with a 4,4′,4″,4‴-tetrasulfophthalocyanine complex of molybdenum oxide.[21] Nevertheless, partial oxidation products of glucose irreversibly adsorb on and poison most electrocatalysts.[22] Hence, electrochemical assays of biological glucose solutions utilize glucose oxidation-catalyzing enzymes.

### 2.2. The Enzymes of Glucose Electrooxidizing Anodes

The two families of enzymes that are most widely used in the electrooxidation of glucose are glucose oxidases (GOx)[23] and PQQ-glucose dehydrogenases (PQQ-GDH). The wild-type enzymes were originally derived, respectively, from *Aspergillus niger* and *Acinetobacter calcoaceticus.* The wild-type enzymes were replaced by engineered enzymes, produced in other organisms. The purpose of their mutation and expression in different organisms was to increase enzyme yield, facilitate enzyme purification, increase specific activity, improve the enzyme stability, and enhance selectivity for glucose.[24–35]

The two enzyme families differ in their redox potentials, the strengths of the bonds between their protein-devoid apoenzymes and their cofactors, their cosubstrates, their turnover rates, their Michaelis constants ($K_m$), and their selectivity for glucose.

The FAD cofactor of GOx is strongly bound to apo-GOx and $FADH_2$-GOx reacts with $O_2$ to yield FAD-GOx and $H_2O_2$. The apparent formal redox potential of GOx at 25 °C at pH 5.3 is $-0.063 \pm 0.011$ V versus SHE; at pH 9.3 it is $-0.200 \pm 0.010$ V versus SHE.[36] Nevertheless, according to a recent re-estimate the apparent formal redox potential of GOx at pH 7.2 is $-0.048$ V versus SHE.[37] GOx is relatively specific for glucose. In the electrochemically relevant half-reaction in which glucose is oxidized by FAD-GOx, about $5 \times 10^3$ glucose molecules are oxidized per second.[15]

PQQ-GDH catalyzes not only the oxidation of glucose, but also of other sugars; the PQQ cofactor is moderately well bound to the apoenzyme in the presence of excess $Ca^{2+}$, which also stabilizes the binding of the PQQ-cofactor by the apoenzyme.[38] Its redox potential at pH 7.0, in the presence of excess $Ca^{2+}$, is $10.5 \pm 4$ mV versus SHE.[38] Unlike the $FADH_2$ of GOx, the $PQQH_2$ of GDH is not oxidized by $O_2$.[39] In the half-reaction of PQQ-GDH, in which glucose is oxidized, 11,800 glucose molecules are oxidized per second.

In addition to PQQ-GDH, two other members of the dehydrogenase family have begun to see application in electrochemical glucose detection. These are NAD-dependent GDH[40] and FAD-dependent GDH.[41] These enzymes combine the oxygen independence of PQQ-GDH with the specificity (toward nonglucose sugars) of GOx, and it is likely that they will be more widely used in the future.

### 2.3. Enzyme-Catalyzed $O_2$-Oxidation of Glucose

In a 1932 study, Otto Warburg and Walter Christian showed that the "yellow enzyme" from yeast was rendered colorless upon reduction by its then still undefined substrate(s), and that its color was restored upon its reoxidation by shaking with gaseous $O_2$.[52] In 1936, Hugo Theorell showed that reaction of the reduced "yellow enzyme" with $O_2$ produced $H_2O_2$. Franke and Deffner isolated in 1939 yellow glucose oxidase (GOx) from *Aspergillus niger* and established that the GOx reaction center contained flavin. They also showed that the glucose-reduced flavin of GOx was oxidized by $O_2$, by cytochrome C, and by quinonoid dyes like toluylene blue, thionine, methylene blue, pyocyanine, and safranine T.[23] The dependence of the Pt electrode potential on $O_2$ partial-pressure was well-known at the time, as was the electrooxidation of $H_2O_2$ on platinum. Thus, the basis for building GOx-based electrodes that could have monitored glucose concentrations potentiometrically through measuring the drop in $O_2$ partial pressure upon its consumption in reactions 1 and 2, or amperometrically, upon the electrooxidation of the $H_2O_2$ produced (reaction 3), existed already in 1939.

$$\text{glucose} + \text{yellow FAD-GOx} \rightarrow \text{gluconolactone} + \text{colorless } FADH_2\text{-GOx} \quad (1)$$

$$\text{colorless } FADH_2\text{-GOx} + O_2 \rightarrow \text{yellow FAD-GOx} + H_2O_2 \quad (2)$$

$$H_2O_2 \rightarrow 2H^+ + O_2 + 2e^- \quad (3)$$

Nevertheless, two decades passed before such electrodes

were built. The first electrochemical glucose assay, based on reactions 1–3, was described in 1961 by Malmstadt and Pardue. They added to reactions 1–3 a fourth reaction, that of molybdate-catalyzed $H_2O_2$-oxidation of $I^-$ to $I_2$ (reaction 4), to enable determination of the $H_2O_2$ concentration by $I^-/I_2$ potentiometry.[43,44]

$$H_2O_2 + 2H^+ + 2I^- \rightarrow 2H_2O + I_2 \quad (4)$$

Five years later, Kajihara and Hagihara, then Makino and Konno, monitored glucose concentrations through $O_2$ consumption by reactions 1 and 2, first without, then in combination with, catalase, decomposing the $H_2O_2$ produced to water and $O_2$ (reaction 5).[45,46]

$$2H_2O_2 \rightarrow 2H_2O + O_2 \quad (5)$$

Next, Updike and Hicks[47,48] significantly simplified the electrochemical glucose assay by immobilizing and thereby also stabilizing GOx. Like Makino and Konno they coupled the GOx membrane with Leland C. Clark's polarographic $O_2$ electrode, the membrane of which enabled its use in biological fluids.[49,50] Note that in presence of catalase ½ of an $O_2$ molecule is consumed per glucose molecule (reaction 6), while in the absence of catalase one $O_2$ molecule is consumed per glucose molecule (reaction 7).

$$2\ \text{glucose} + O_2 \rightarrow 2\ \text{gluconolactone} + 2H_2O \quad (6)$$

$$\text{glucose} + O_2 \rightarrow \text{gluconolactone} + H_2O_2 \quad (7)$$

## 2.4. Enzyme-Catalyzed Redox Couple-Mediated Electrooxidation of Glucose

Direct electron tunneling from the $FADH_2$ of GOx to an electrode is much too slow, because the $FADH_2$ is buried at a depth of about 13–15 Å below the electrode-contacting periphery of its glycoprotein.[51] This is the case even when the enzyme is uniquely oriented to minimize the distance between one of its two $FADH_2$-centers and the surface of an electrode. For a 13–15 Å tunneling-distance the tunneling rate is much slower than the rate of glucose oxidation by FAD-GOx, even when the glucose concentration is much less than its physiological 4–8 mM concentration in blood and other tissues of nondiabetic people. In fact, the rate of FAD-GOx catalyzed glucose electrooxidation is too slow to be measured in the absence of redox mediators.

Warburg and Christian discovered in 1932 not only that the reduced "yellow enzyme" is oxidized by $O_2$, but also that it is oxidized, even more rapidly, by the quinoid dye methylene blue.[52] Franke and Deffner similarly showed, in 1939, that the glucose-reduced flavin of GOx, which they discovered, was oxidized not only by $O_2$, but also by cytochrome C, and by quinoid dyes such as thionine, methylene blue (MB), pyocyanine, and safranine T.[23] Again, their observations could have opened the way to redox-couple mediated electrooxidation of glucose (reactions 8 and 9).

$$FADH_2\text{-GOx} + \text{blue MB} \rightarrow \text{FAD-GOx} + \text{colorless } MBH_2 \quad (8)$$

$$\text{colorless } MBH_2 \rightarrow \text{blue MB} + 2H^+ + 2e^- \quad (9)$$

It was, however, 30 years later, in 1970, that Silverman and Brake[53] described redox-couple mediated electrooxidation of glucose. They showed that MB/$MBH_2$, 2,6-dichloroindophenol, indigo disulfonate, phenosafranin, and phenazine methosulfate effectively mediated the electrooxidation of glucose by oxidizing glucose-reduced GOx (reaction 8), that is, $FADH_2$-GOx, and by being electrooxidized (reaction 9).[53]

In many of the studies, but not in the actual home blood-glucose monitoring strips in use, GOx or PQQ-GDH was immobilized on electrodes, most often gold or vitreous carbon, or was immobilized within carbon pastes.[54–85] In other studies the enzyme was deposited as an organized electrocatalytic multilayer,[86–90] to facilitate the modeling of the transport of glucose and the mediator.[86,87] Theoretical models now fully account for the glucose concentration-dependence, the pH dependence, the redox-mediator concentration-dependence and the GOx loading-dependence of the glucose electrooxidation current of electrodes on which GOx is immobilized.[91,92]

### 2.4.1. Organic Mediators

Examples of organic mediators include quinoid dyes,[70,93–97] quinones,[54–56,98–102] oxidized viologens[103–107] and quinone and quinone derivatives,[98,100,101,108–112] including polymeric quinones.[102,113–115] All oxidize glucose-reduced GOx. Mediating quinones have also been synthesized in situ by tyrosinase-catalyzed oxidation of phenols.[116,117] Among the quinoid dye mediators,[118] methylene blue has been the longest studied.[15,16,23,53,119] Measurements of the bimolecular rate constants for the electron transfer from reduced glucose oxidase to oxidized members of this family of mediators provided the exemplary values of $1.6 \times 10^4\ M^{-1}\ s^{-1}$ for thionine, $4.0 \times 10^2\ M^{-1}\ s^{-1}$ for brilliant cresyl blue, $9.8 \times 10^2\ M^{-1}\ s^{-1}$ for azure A, $9.0 \times 10^3\ M^{-1}\ s^{-1}$ for daunomycin, and $1.2 \times 10^6\ M^{-1}\ s^{-1}$ for dopamine.[110] A drawback of some, but not all, quinones is their reaction with cysteine and other protein residues, which destabilizes the mediator-enzyme systems.[120–122]

The bimolecular rate constants of the steady-state oxidation of GOx by phenothiazines, phenoxazines, Wurster's salts, dithia- and tetrathia-aromatic compounds, measured at pH 7.0, vary between $10^3$ and $10^8\ M^{-1}\ s^{-1}$. For phenothiazines, phenoxazine and Wurster's salts the rate constants depend on the redox potential, and vary according to the outer sphere electron transfer theory of Marcus and Sutin.[123] The rate constants of some of the substituted thiaaromatic compounds with the GOx reaction channels or centers differ, however, from those predicted by the classical electron transfer theory.[124]

Glucose electrooxidation, catalyzed by GOx and heterocyclic dihydropolyazines such as 5,10-dihydro-5,10-dimethylphenazine and 1,4-dihydro-1,3,4,6-tetraphenyl-s-tetrazine adsorbed on graphite, has been attributed to the radical cation of the dihydropolyazines.[94] *N,N′*-Di(4-nitrobenzyl)viologen dichloride, poly(o-xylylviologen dibromide), and poly(p-xylylviologen dibromide) mediate electron transfer from $FADH_2$-GOx to carbon paste electrodes, whereas the redox potentials of other viologens are too reducing to oxidize $FADH_2$-GOx.[104]

Quinones and quinoid dyes (Q) also catalyze the electrooxidation of NADH generated when $NAD^+$-GDH is reduced by glucose (reactions 10–12).[40,125–129] Of these, 1,10-phenanthroline quinone is in use in glucose monitoring strips.[130]

$$\text{glucose} + \text{NAD}^+\text{-GDH} \rightarrow \text{gluconolactone} + \text{NADH-GDH} + \text{H}^+ \quad (10)$$

$$\text{NADH-GDH} + \text{Q} \rightarrow \text{NAD}^+\text{-GDH} + \text{QH}_2 \quad (11)$$

$$\text{QH2} \rightarrow \text{Q} + 2\text{H}^+ + 2\text{e}^- \quad (12)$$

### 2.4.2. Inorganic Mediators

The $O_2/H_2O_2$ couple itself can be considered as the natural mediator for GOx. It is, however, far from being the optimal mediator of GOx electrooxidation, because of the low (~0.2 mM) solubility of $O_2$ in physiological solutions at ambient temperature. Mediation by the hexacyano-complexes of iron,[131,132] cobalt, and ruthenium has been extensively studied.[133–135] Of these, the $Fe(CN)_6^{3-/4-}$ couple is used in home blood-glucose monitoring strips.

### 2.4.3. Metal–Organic Mediators

The redox potentials of complexes of pentacyanoferrate(III) with pyridine, pyrazole, imidazole, histidine, and aza- and thia-heterocycles or benzotriazole, benzimidazole, and aminothiazole as their sixth ligand span the potential range from 300 to 470 mV versus SHE at pH 7.2. Study of their rates of oxidation of $FADH_2$-GOx established that the parameters controlling the rate constants included the mediator's self-exchange rate constant, its charge, and its steric fit into and the binding by the GOx reaction center.[37]

Families of metal organic redox mediators actually used in blood-glucose monitors include ferrocene-derivatives and $Os^{2+/3+}$-complexes.[136–138] Because Exactech, the first electrochemical home blood-glucose monitor introduced by Genetics International/Medisense, utilized PQQ-GDG and a ferrocene-derivative,[139] ferrocenes were extensively studied as electron shuttling mediators between both GOx and PQQ-GDH and electrodes.[140–146] Ferrocene is small enough to penetrate the reaction channel of GOx.[147] Mediation rates were determined for 42 ferrocene-derivatives and explained by their redox potentials and structure. Their bimolecular rate constants for $FADH_2$-GOx oxidation range from $3 \times 10^4$ to $8 \times 10^6$ $M^{-1}$ $s^{-1}$.[148] The most intensively studied ferrocene-derivatives are ferrocenemethanol[88,90,91,149–161] and ferrocenecarboxylic acid.[155,162–164] Several of the ferrocenes meet the requirements for application in blood-glucose analyzers, which include high solubility in water, fast electron-shuttling, stability, and pH-independence of the redox potential. One of the best mediating ferrocenes for $FADH_2$-GOx electrooxidation is 1,1′-dimethyl-3-(2-amino-1-hydroxyethyl) ferrocene, the mediator of the MediSense ExacTech and Precision QID blood-glucose meters.[165–167]

Nickelocene, adsorbed on pyrolytic graphite, has a redox potential of 115 mV vs SHE. It also mediates the electrooxidation of $FADH_2$-GOx at 220 mV.[168] Manganese cyclopentadienyl (Cp) half-sandwich complexes are comparable in their electron-shuttling rates to ferrocene derivatives, an exemplary bimolecular rate constant, of $[(h\text{-}MeC_5H_4)Mn(NO)(CN)_2]Na$, being $2.1 \times 10^5$ $M^{-1}$ $s^{-1}$.[167] The chromium half-sandwich complex $[h\text{-}C_6Me_4(NH_2)_2Cr(CO)_3]$, the redox potential of which is +270 mV versus SHE, displays reversible electrochemistry but is a relatively slow mediator of $FADH_2$-GOx electrooxidation.[167]

The redox potentials of tris-(4,4′-substituted-2,2′-bipyridine) complexes of the group VIII metals $Fe^{2+/3+}$, $Ru^{2+/3+}$, and $Os^{2+/3+}$ range from −100 to +840 mV versus SHE and their bimolecular rate constants for $FADH_2$-GOx oxidation reach $10^7$ $M^{-1}$ $s^{-1}$. $Os^{2+/3+}$ complexes with amino substituted bipyridines and tris-(4,4′-dimethoxy-2,2′-bipyridine) have particularly high rate constants, suggestive of their binding within the GOx reaction channel or reaction center.[138] The bimolecular rate constants for $FADH_2$-GOx oxidation by substituted-1,10-phenanthroline complexes of $Ru^{2+/3+}$ and $Os^{2+/3+}$ are of $10^6$–$10^7$ $M^{-1}$ $s^{-1}$ when the substituents are electron-donors.[169] *cis*-$[Ru(LL)_2XY]^{n+}$ complexes (LL = 2,2′-bipyridyl (bpy), 1,10-phenanthroline, and 4,4′-dimethyl-2,2′-bipyridyl (X, $Y = Cl^-, Br^-, CO_3^{2-}, NO_2^-, SCN^-, N_3^-, H_2O$, and DMSO) mediate electrooxidation of $FADH_2$-GOx.[170] Cyclometalated $Ru^{2+/3+}$ compounds are also efficient mediators of $FADH_2$-GOx electrooxidation.[171]

## 2.5. Electrical Wiring of GOx by Electron-Conducting Redox Hydrogels

The mediating redox couples can be freely diffusing, protein-bound,[172–174] or bound to a peripheral oligosaccharide of GOx via a long, typically 8–13 atom, spacer arm.[175] Redox hydrogels constitute, however, the only known electron-conducting phase in which glucose, gluconolactone, and water-soluble ions dissolve and diffuse. Redox hydrogels, in which GOx is immobilized, catalyze the electrooxidation of glucose.[176] Unlike the electrocatalysts formed of GOx or PQQ-GDH and diffusing redox mediators, the glucose-oxidation catalyzing redox-hydrogels have no leachable constituents. Their redox centers are tethered to the insoluble, but water-swollen, cross-linked polymer-network of the gel.[177–179] Because the redox hydrogels envelope the redox enzymes, they electrically connect the enzymes' reaction centers to electrodes irrespective of the spatial orientation of the enzyme at the electrode surface and also connect multiple enzyme layers. Hence, the attained true current densities are usually about 10-fold higher, and in some cases 100-fold higher, than they are when enzyme monolayers are packed onto electrode surfaces and when most of their redox centers are electrically connected to the electrode surfaces. Specifically, the current densities of glucose electrooxidation on smooth, nonporous, electrodes exceed 1 mA $cm^{-2}$ already at 0.0–0.1 V versus Ag/AgCl.[177–180]

### 2.5.1. Mechanism of Electron-Conduction in Redox Hydrogels

Redox hydrogels conduct electrons by self-exchange of electrons or holes between rapidly reduced and rapidly oxidized redox functions tethered to backbones of cross-linked polymer networks. Although the networks, which are formed by cross-linking of water-soluble redox polymers, swell in water, they do not dissolve. The redox polymers conduct electrons, or holes, through self-exchange in the water swollen hydrogels.[181] The self-exchange results from Marcus-type collisional electron transfer,[123] which physicists know as phonon-assisted tunneling. Here a reduced redox-species collides with an oxidized redox-species, the reduced species transferring its electron, or the oxidized species transferring its hole. Although, in theory, electrons or holes could also propagate by hopping between fixed-position redox centers,[182] trap-to-trap hopping of solid state physics is rarely seen in redox hydrogels.

Because electron transfer by self-exchange requires collisions between reduced (electron-loaded) and oxidized (hole-loaded) redox centers,[181,183] electron diffusion slows when

an overwhelming majority of the redox centers are either oxidized or reduced. Thus, the electronic conduction is poor when the hydrogel is poised at a potential far positive or far negative of its redox potential. It is highest when the density of reduced and oxidized centers is about equal, that is, when the hydrogel is poised at its redox potential. The rate of self-exchange of electrons or holes decays exponentially with distance. It is fastest when the redox functions are tethered to the cross-linked polymer networks by long and flexible spacers which are, optimally, between 10 and 15 atom long.[178,180] The long and flexible spacers increase the amplitude of the displacement of the tethered redox centers. They enable thereby effective electron-transferring collisions, even when the time-averaged distance between the oxidized and the reduced redox centers is between 1 and 3 nm, that is, when the concentration of the tethered redox centers in the fully swollen hydrogel is between 1 and 0.1 M.

The apparent electron diffusion coefficients, $D_e$, of the redox hydrogels depend on, and are predominantly determined by, the segmental mobility, which increases with hydration and decreases upon excessive cross-linking.[181] Increasing the cationic charge of the redox polymer backbone, either by quaternizing part of the pyridines of a poly(4-vinylpyridine)-based redox polymer, or by heavy coordination of the poly(4-vinylpyridine) with $[Os(bpy)_2Cl]^{2+/3+}$, ensures adequate hydration at any pH, and provides a $D_e$ of $3.9 \times 10^{-8}$ cm$^2$ s$^{-1}$.[183]

Charge neutrality is maintained in any volume element of the hydrogel that contains a redox center. Hence, either anions diffuse upon the transfer of an electron from a reduced to an oxidized redox center in the direction opposite to that of the movement of the electron, or cations diffuse in the direction of the movement of the electron,[184] or both anions and cations diffuse in their respective directions. For this reason, the value of $D_e$ may approach, but can never exceed, the diffusion coefficient of the most rapidly diffusing anion or cation present.[185] This limiting value is closely approached when the tethers binding the redox centers to the redox-polymer backbone are long and flexible. For a 13 atom-long flexible tether, $D_e$ reaches $5.8 \times 10^{-6}$ cm$^2$ s$^{-1}$.[178]

### 2.5.2. Mechanism of Direct Glucose Electrooxidation

Catalysis of the direct electrooxidation of glucose in involves (a) transfer of electrons (and protons) from glucose to FAD reaction centers of glucose oxidase, which are reduced to $FADH_2$ (b) transfer of electrons from the $FADH_2$ centers to the "wiring" electron-conducting hydrogel and (c) transport of electrons through the hydrogel to the electrode. The second and third steps are enabled by motion of tethered segments of the cross-linked redox polymer network, allowing the redox centers to approach each other sufficiently ("collide") to transfer electrons or holes. Through such collisions, electrons flow from the protein-buried $FADH_2$ centers to redox centers of the polymer network. The collisions also lead to exchange of electrons between reduced and oxidized centers of the network, which is the underlying cause of the electron conduction by the water-swollen networks. Thus, hydration of the cross-linked redox polymer has two effects, both essential for glucose-electrooxidation. It enhances the movement of segments, which are, in the absence of hydration, tightly held by electrostatic (ionic and dipolar) interactions, and it makes the cross-linked polymer permeable to the water-soluble reactant, glucose, and to gluconolactone, the electrooxidation product. Hydration, and with it the electron diffusion coefficient, generally increases with the charge density on the polymer in the wired enzyme systems.[176,186]

### 2.5.3. Organic and Metal−Organic Redox Centers in Electron-Conducting Hydrogels

The redox centers of the hydrogels can be organic or metal-organic. An example of a hydrogel with organic redox centers is the hydrogel formed upon reacting templated, linear-chain, polyaniline, cross-linked and made hydrophilic by a water-soluble, extended chain diepoxide, like poly(ethylene glycol) diglycidyl ether.[187]

When GOx is immobilized in such an organic polymer-based hydrogel, the hydrogel catalyzes the direct electrooxidation of glucose.[187]

The most extensively studied direct glucose electrooxidation catalyzing redox hydrogels are, made however, of GOx and water-soluble polycationic polymers, like poly(4-vinylpyridine), poly(N-vinylimidazole) or poly(acrylamide)-copoly(N-vinylimidazole), with tethered complexes of $Os^{2+/3+}$. The $Os^{2+/3+}$ ligands are typically substituted or unsubstituted pyridine (py), 2,2′-bipyridine (bpy),[188–194] di-*N*-alkylated-2,2′-di-imidazole.[178]

### 2.5.4. Mechanical Properties: Balancing the Strength against the Electronic Conductivity

Because the redox hydrogels are formed of water-soluble polymers, their mechanical properties, ranging from soft jellies to tough, leather-like materials, are defined by the molecular mass of the starting polymer and by the extent of cross-linking. And because the segmental mobility, on which the electron diffusion depends, increases with hydration, and because cross-linking limits hydration and thus segmental mobility, mechanical strengths and high apparent electron diffusion coefficients are difficult to achieve simultaneously. They are, nevertheless, simultaneously achieved when the tethers are long and flexible because, in this case, even if the backbones are highly cross-linked, the redox functions at the ends of the tethers can still swing and exchange electrons.

The shear strength of the redox hydrogel films on rotating disk electrodes is conveniently measured by determining the angular velocity at which a drop in the voltammetric peak is first observed.[195] The shear stress, $\tau$, resulting from the rotation, is $\tau = 0.616\rho^{1/2}\Omega^{3/2}r$. Here $\rho$ is the density of the solution; $\nu$ is its kinematic viscosity, $\mu/\rho$; $\mu$ is the viscosity of the solution; $\Omega$ is the rotation rate; and $r$ is the distance from the center of the rotating disk. Above a critical angular velocity, the part of the hydrogel closest to the rim of the rotating electrode is sheared off, and a drop in the current is observed. Adequately cross-linked redox hydrogels withstand shear stresses of $10^{-2}$ N/m$^2$, but are sheared off above 0.1 N/m$^2$.[195] Water-soluble cross-linkers, with reactive functions separated by long and flexible spacers, such as 400 Da polyethylene glycol diglycidyl ether, are preferred for the mechanical strengthening of the hydrogels.[195] Films of some of the $Os^{3+}$ complex-containing redox hydrogels can, however, also be cross-linked by ligand exchange.[196]

### 2.5.5. Electrodeposition of Glucose Electrooxidation-Catalyzing Electron-Conducting Hydrogels by Ligand Exchange

While inner coordination sphere halides, for example, chlorides, are not exchanged in $Os^{3+}$ complexes, where they are electrostatically strongly bound, they are exchanged by pyridine, imidazole, or primary amine functions if the complex is of electroreduced $Os^{2+}$, where the electrostatic bond is weaker. Thus, when an electrode is densely covered by adsorbed redox polymer and the $Os^{3+}$ is electroreduced to $Os^{2+}$, inner coordination sphere halides of one strand are exchanged by backbone pyridine, imidazole, or primary amine functions of proximal adsorbed strands, coordinatively binding the two strands. In effect, the polymer is electrodeposited from its aqueous solution by the reductive cross-linking.[196] Because GOx is a polyanion and forms an electrostatic adduct with the electrodeposited redox polymer, and because the protein of GOx has ligand-exchanging amines at is periphery, the redox hydrogel and GOx can be coelectrodeposited. The electrodeposited films catalyze the electrooxidation of glucose.[196–198]

The shelf life of concentrated solutions of the redox polymers that can be electrodeposited by ligand exchange is short when the oxidized redox centers are reduced by a codissolved organic constituent, the reduction causing now unwanted cross-linking and precipitation. Solutions and hydrogels of redox polymers having complexes that cannot exchange ligands are, however, stable. For example, solutions and hydrogels comprising $Os^{3+}$ complexes with six heterocyclic nitrogen ligands, such as tethered $Os(bpy)_3^{2+/3+}$, are particularly stable.

### 2.5.6. Redox Potentials of the Electron Conducting Hydrogels

The redox potentials of the electron conducting hydrogels are defined primarily by the transition metal ion of their complex and by its ligands.[199] The reported redox potentials of $Os^{2+/3+}$ comprising hydrogels range from about −0.2 V vs Ag/AgCl (for the tris *N*,*N*′-dialkylated-2,2′-diimidazole complex based gels)[178,180] to +0.55 V vs Ag/AgCl (for the (4,4′-dimethyl-2,2′-bipyridine)$_2$(4-aminomethyl-4′-methyl-2,2′-bipyridine)]$^{2+/3+}$ complex comprising gels).[194,200] Because the half-cell potential for the exemplary electrode reaction $[Os(bpy)_3^{3+}][Cl^-]_3 + e^- \leftrightarrow [Os(bpy)_3^{2+}][Cl^-]_2 + Cl^-$ is chloride anion concentration dependent, the concentration of chloride (or of other anions) in the hydrogels affects the redox potential. When the cross-linked redox polymer is a polycation, for example, partly quaternized poly(4-vinylpyridine) (PVP), the chloride concentration in the hydrogel can be as high as about 1 M when in equilibrium with a physiological solution, in which the chloride concentration is only 0.14 M. Hence, the redox potential is upshifted by about 50 mV. Because the density of cationic sites increases when the redox polymer is cross-linked and water is squeezed out, excessive cross-linking also upshifts the redox potential, though the shift is typically small, only 10–20 mV.

### 2.5.7. Charge of the Polymer Backbones of the Electron Conducting Hydrogels

The GOx-wiring redox hydrogels are tailored to be polycations, to avoid partial phase separation from glucose oxidase, which is a polyanion at physiological pH.[201] The redox center of the exemplary polymer **I** of Figure 1 is tailored to have a redox potential of −0.20 V vs Ag/AgCl, just slightly oxidizing relative to the FAD/FADH$_2$ center of glucose oxidase.

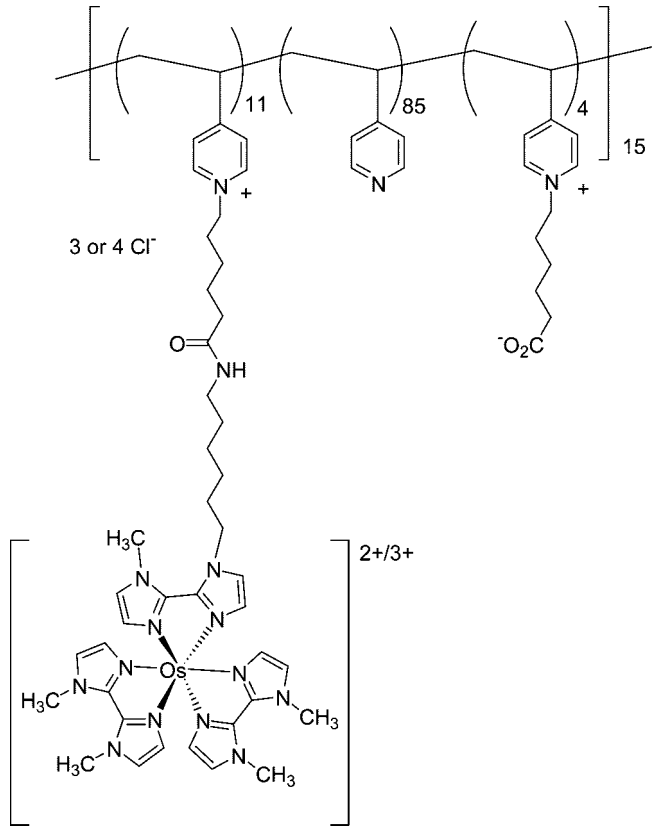



**Figure 1.** Structure of polymer **I**, a redox polymer designed to electrically connect the reaction centers of glucose oxidase to electrodes. A 13 atom long tether binds the redox center to the poly(4-vinylpyridine) backbone, which is partially quaternized to make the polymer–water soluble. The apparent electron diffusion coefficient of the redox hydrogel formed upon cross-linking the polymer with polyethylene glycol diglycidyl ether and hydration is $5.8 \times 10^{-6}$ cm$^2$ s$^{-1}$, and its redox potential is −0.2 V vs Ag/AgCl. The functions bound to the poly(4-vinylpyridine) backbone are randomly distributed.[178,180]

### 2.5.8. Applications of Glucose Oxidation Electrocatalysts Based on Electron Conducting Redox Hydrogels

The electron conducting redox hydrogels serve to electrically connect the redox centers of enzymes to electrodes, enabling their use whenever leaching of electron-shuttling diffusional redox mediators must be avoided. This is the case in glucose concentration monitoring electrodes implanted in diabetic people,[202,203] and in membrane-less biofuel cells, the anodes and cathodes of which would be shorted if the mediator could diffuse, and in flow cells for the electroanalysis of glucose.

FreeStyle Navigator of Abbott Diabetes Care of Alameda, CA[204] is a glucose monitoring system for diabetes management, measuring and transmitting the glucose concentration to a PDA-like device about every minute. Its core component, a disposable, miniature subcutaneously implanted amperometric glucose sensor, comprises redox hydrogel-wired glucose oxidase.[203] Comparison of 20,362 measurements of glucose with the Navigator Continuous Glucose Monitoring System in the interstitial fluid, with measurements of venous blood glucose with the Yellow Springs Instrument laboratory

reference glucose analyzer, showed a median absolute relative difference (ARD) of 9.3%. The percentage of the FreeStyle Navigator measurements that were in the clinically accurate Clarke error grid A zone was 81.7% and the percentage in the benign error grid B zone was 16.7%. In the first of the recommended 5 days of its use 82.5% of the measurements were in the A zone, and on the 5th day 80.9% were in the A zone.[205] The clinical performance of the FreeStyle Navigator Continuous Glucose Monitoring System in children and adults has been analyzed and reported.[206–209]

### 2.6. Metal-Particle GOx-Plug Relay Based Glucose-Electrooxidation Catalysts

In 2003 Xiao et al. reconstituted apo-glucose oxidase using FAD bound to 1.4-nm gold nanocrystals showing that it is possible to electrooxidize glucose when the electrons are relayed to an electrode via a gold-nanoplug in the GOx. The contact to the electrodes was, however, poor and glucose was electrooxidized only at high overpotentials. Current densities were not reported.[210] In 2007 Dagan-Moscovich wired GOx, by modifying the periphery of the enzyme through its reaction with poly(glutaraldehyde), reacting the aldehyde-functions with alanine and reducing by the resulting Schiff-bases $Ag^+$ to form silver nuclei, on which more silver was precipitated. The $Ag^+$ ions did not substantially affect the functioning of GOx.[211] Again, the overpotential was high and the current density was not reported.

## *3. Electrochemical Monitoring of the Glucose Concentration by Its GOx-Catalyzed $O_2$-Oxidation*

### 3.1. $O_2$-Depletion Monitoring upon GOx-Catalyzed $O_2$-Oxidation of Glucose

The concentration of glucose can be monitored through the GOx-catalyzed oxidation of glucose by $O_2$ in combination with amperometric monitoring of the rate of decline of the solution $O_2$-concentration (reaction 6 in the presence of catalase, or reaction 7 in its absence). The $O_2$ concentration has been monitored either with a Pt electrode or with an indium tin oxide (ITO) electrode, coated with a polymer that was highly $O_2$ permeable and sufficiently proton-permeable, so as to allow reaction 13 or 14, following, respectively, reaction 6 or 7.

$$O_2 + 4H^+ \rightarrow 2H_2O \quad (13)$$

$$O_2 + 2H^+ \rightarrow H_2O_2 \quad (14)$$

Rigorous chemical engineering modeling of glucose sensors based on amperometric $O_2$ monitoring pointed to their performance limits and preferred, but usually difficult to reproducibly manufacture, structures. The parameters affecting their dynamic range, response time, and sensitivity include the membrane thickness, the $O_2$ permeability of the membrane, the thickness of the GOx containing film, and the specific activity and loading of the GOx, which have been related through the Damköhler and Biot numbers and the Thiele $O_2$ moduli.[212,213] Miniature, low drift and low cost sensors, made by techniques used in the manufacture of integrated circuits and their interconnects, reaching 90% of the ultimate current in about a minute and responding in the 20 $\mu$M to 1.4 mM glucose concentration range, have been designed.[214–219] Amperometric probes with transparent and flexible ITO electrodes, monitoring glucose concentrations in the 60 $\mu$M to 1.2 mM range were also reported.[220] Furthermore, the high $O_2$-solubility in, and the resultant high $O_2$-permeability of, elastomeric silicone was exploited in a poly(dimethylsiloxane)-based carbon-paste glucose sensor, which allowed glucose assays at concentrations as high as 40 mM, with linearity maintained up to 20 mM.[221]

### 3.2. Electrooxidation of the $H_2O_2$ Produced upon Enzyme-Catalyzed $O_2$-Oxidation of Glucose

The assay of glucose through enzyme-catalyzed reactions in which $H_2O_2$, the product of reactions 1 and 2, is amperometrically monitored has been the subject of 400 publications. In most, the catalyst of the $O_2$ oxidation of glucose has been GOx, although the use of pyranose oxidase[222] also has been explored. The amperometric $H_2O_2$ assay is carried out in one of three formats: (a) catalytic electrooxidation the $H_2O_2$ at +0.3–0.8 V vs SCE, commonly at about 0.6 V vs SCE; (b) catalytic electroreduction of the $H_2O_2$, typically near −0.1 V vs SCE; or (c) $H_2O_2$-oxidation of a peroxidase, usually horseradish peroxidase, followed by mediated or direct electroreduction of the oxidized peroxidase.

Although Pt on graphite is most often used as the $H_2O_2$ electrooxidation catalyst, other catalysts and substrates have been studied. The catalyst-substrates[223,224] studied include carbon pastes, highly oriented pyrolytic graphite, diamond, carbon nanotubes, and conducting polymers, such as polypyrrole and polyaniline. The nonplatinum based $H_2O_2$ electrooxidation catalysts include palladium, nickel cyclam, ruthenium, ruthenium−platinum alloys, iridium dioxide, single-walled carbon nanotubes and polypyrrole functionalized multiwalled-carbon nanotubes.[62,225–235] Part of the catalyst research has been aimed at reducing the potential at which the $H_2O_2$ is electrooxidized, that is, at better selectivity in the presence of electrooxidizable interferants.[236] $H_2O_2$ has been typically electrooxidized on Pt about 600 mV vs SCE, where ascorbate, urate, and acetaminophen are also electrooxidized. When present, they were excluded through the use of cation exchange or other permselective membranes.

### 3.3. Electroreduction of $H_2O_2$ Produced upon Enzyme Catalyzed $O_2$-Oxidation of Glucose

The $H_2O_2$-electroreduction catalysts studied include Pd, Pd−Pt, Pt-nanowires; Au-nanoparticles distributed in a porous silicate; DNA-$Cu^{2+}$; DNA-$Ag^+$; $Cu^{2+}$, $Fe^{2+}$; $Zn^{2+}$ and $Ce^{3+}$-modified silicate xerogels and $Co(CN)_6^{3-/4-}$-chitosan modified carbon nanotubes.[237–244] Miniaturization of glucose sensors based on $H_2O_2$-electroreduction provided for their implantation and integration in arrays.[245–247] Membranes improving their selectivity for glucose and extending their dynamic range have been described.[248]

#### *3.3.1. Peroxidase-Catalyzed $H_2O_2$ Electroreduction*

Amperometric assay of the $H_2O_2$ produced in reactions 7 or 13 through its oxidation of a peroxidase,[163,236,249–261] (reaction 15, where $HRP^{2+}$ is Fe(IV)-HRP, that is, peroxidase with four-valent iron, followed by mediated (reactions 16, 17)[163,262,263] or direct electroreduction[125,250] of the peroxidase, has been widely studied. The peroxidase most commonly used is horseradish peroxidase (HRP), though other peroxidases, such as thermostable soybean peroxidase,[196,264–266] have also been used.

$$H_2O_2 + HRP \rightarrow HRP^{2+} + 2OH^- \quad (15)$$

$$HRP^{2+} + 2M^{n+} \rightarrow HRP + 2M^{(n+1)+} \quad (16)$$

$$2M^{(n+1)+} + 2e^- \rightarrow 2M^{n+} \quad (17)$$

### 3.4. Monitoring the Drop in pH upon Enzyme Catalyzed $O_2$-Oxidation of Glucose with Field Effect Transistors

Because $H^+$ is released in the electrooxidation of $H_2O_2$ (reaction 3), glucose concentrations can be monitored by measuring the pH, as long as the influx of glucose and the out-flux of protons are well-defined. Hydrolysis of gluconolactone, the oxidation product of GOx or NADH-GDH catalyzed reaction of glucose and oxidants, produces gluconic acid. When the gluconolactone generated (reactions 5 and 7) is rapidly hydrolyzed to gluconic acid, the glucose concentration can be similarly monitored through the associated pH change. Potentiometric glucose monitoring is, however, rarely practiced because of the many parameters affecting the local pH and because the scaling of the signal with the logarithm of the $H^+$ concentration, rather than its scaling linearly, as is the case in amperometric monitoring. Thus, when the rates of generation and neutralization of the acid are known, the concentration of glucose can be related to the local pH at a pH-sensitive device, usually an unencapsulated field effect transistor (FET), referred to as a CHEMFET, proton-sensitive field-effect transistor, ion-sensitive FET (ISFET), or enzyme-FET (ENFET), all of which are usually made of silicon. Here the current between their source and drain is a function of the surface density and type of charge of the adsorbed ions, hence the pH sensitivity. Typically, the currents of two matched FETs are compared, with GOx immobilized on one and a noncatalytic protein on the other.[267–281]

## *4. Central Laboratory and Desktop Glucose-Analyzers*

### 4.1. The First Central Laboratory Glucose-Analyzers

On the basis of the work of Arnold H. Kadish and his colleagues, Beckman Instruments of Fullerton, CA, introduced in 1968 the first commercially available clinical glucose analyzer.[282,283] In the Beckman glucose analyzer, GOx was added to the analyzed sample, and the decrease in dissolved $O_2$ concentration was monitored (see section 3.1), initially with the Beckman Polarographic Oxygen sensor electroreducing $O_2$ on a gold cathode, then with the polarographic $O_2$-electrode of Leland C. Clark Jr.[284]

The YSI model 23 glucose analyzer was introduced by Yellow Spring Instruments (Yellow Springs, Ohio) in 1974. This model and later YSI glucose analyzers served historically as gold standards with which the accuracy of other glucose analyzers was compared. Even though Hardy Trolander, founder of YSI, and Clark were closely associated, the model 23 and the later YSI glucose analyzers assayed, unlike the Beckman glucose analyzer, not the $O_2$ consumed, but the $H_2O_2$ produced in reaction 2 by its electrooxidation on Pt. The core of the YSI Model 23 and of later YSI glucose analyzers was its glucose monitoring probe, which had two polymer layers and an inner Pt electrode, on which the $H_2O_2$ was electrooxidized to $O_2$ (reaction 3). The membrane contacting the analyzed solution, which was 30 times diluted when blood or serum was analyzed, was made of polycarbonate. Other than preventing fouling, this membrane reduced the glucose influx, extending thereby the dynamic range and the useful life of the probe. The GOx, which catalyzed the glucose conversion, that is, the generation of $H_2O_2$, was immobilized on the Pt-side of this outer polycarbonate membrane. The inner membrane was made of cellulose acetate. It excluded most interferents, but was permeable to $H_2O_2$, allowing its diffusion to the Pt electrode.

The YSI glucose analyzers introduced two significant design principles, which were adopted in later flow-analyzers and in implantable glucose electrodes. First, the influx of glucose was reduced (by dilution of the analyzed solution and by the outer polycarbonate membrane) sufficiently for all of the glucose passing the outer membrane to react with dissolved $O_2$, even though the concentration of $O_2$ in water is at saturation only about 0.2 mM at 25 °C, while the glucose concentration in the undiluted blood of a diabetic patient can be 30 mM, 150 times higher. Second, reduction of the glucose influx and immobilization (through glutaraldehyde cross-linking) of an initially large excess of GOx on the Pt-side of the outer membrane ensured complete conversion of the glucose that passed the outer membrane, even after most of the initial GOx activity was lost. This extended the operational life of the probe, which maintained linear increase of its output current with glucose concentration as long as all of the glucose influx was converted.

### 4.2. Contemporary Central Laboratory Electrochemical Glucose Analyzers

The current YSI 2300 STAT glucose analyzer utilizes a 25 $\mu$L sample, has a throughput one sample per 100 s, measures glucose concentrations up to 50 mM, and its error is the larger of $\pm$2% or 0.2 mM. The working life of its membrane is 21 days.

Eppendorf-Netheler-Hinz GmbH of Hamburg, Germany, and PGW Prüfgeräte-Werk Medingen, Germany, introduced amperometric $H_2O_2$ electrooxidation based clinical glucose analyzers in 1986. These analyzers were followed by those of EKF Diagnostic GmbH, Magdeburg, Germany, and of CARE Diagnostica GmbH Voerde, Germany. All use a GOx-comprising membrane, made by BST Bio Sensor Technologie GmbH Berlin, Germany. They combine periodically replaced GOx membranes with permanent, built in, Pt-based $O_2$ and Ag/AgCl electrodes. The BST GOx membranes are designed to provide selectivity for glucose, low drift, and long operational life. Furthermore, for the recent CARE Diagnostica systems, as well as those of other companies, BST introduced and now manufactures a sensor that is long-lived and comprises the GOx-containing membrane, a Pt$-O_2$ electrode and a Ag/AgCl electrode, all integrated on a ceramic substrate. The novel BST membranes and sensors are so stable that they are replaced only a few times a year.

Unlike in the Beckmann or YSI systems, which measure the approach of a current plateau, the Eppendorff, CARE, and Voerde systems measure the first derivative of the $H_2O_2$ electrooxidation current, which allows completion of the assays in less than 5 s. The blood samples used are diluted 50-fold, the dilution disrupting the red blood cells, interrupting their glycolysis. Because of the interruption of glycolysis, the diluted blood samples are stable for at least 24 h, contributing to the accuracy of the assays and making these convenient for use by central hospital and clinical

laboratories, allowing efficient usage when many blood samples arrive in a narrow time-window and a few samples arrive in others.

A&T Co. of Kanagawa, Japan, produces a GOx/$O_2$ electrode based glucose analyzer for hospital and central laboratory usage requiring about 30 $\mu$L of blood or serum, with a throughput of about 200 samples/h, providing about $\pm$0.3 mM reproducibility.

### 4.3. Hand-Held Electrochemical Glucose-Analyzers for Hospital Wards, Emergency Rooms, and Physician's Offices

In 2004 BST introduced the first hand-held, relatively low-cost electrochemical clinical glucose analyzer (its Glukometer 3000), made with a biosensor required replacement only after 30 days of use or after assay of as many as 1000 samples. Following assay of a whole blood sample, the fluidics of the BST analyzer allows its cleaning with a few microliters of rinsing solution, reducing the biohazardous fluid-disposal burden. Glucose flux reduction and selectivity for glucose are provided by a film topping of the GOx layer, obviating the need for YSI's inner $H_2O_2$-selective polymer layer on the $H_2O_2$ electrooxidizing Pt electrode. The glucose concentrations measured are 0.5–33.3 mM. The system is used in wards of hospitals, emergency rooms, and physician's offices, filling, at low cost, the gap between large central laboratory glucose analyzers and single-use strip based home blood-glucose monitors, used by self-monitoring diabetic people.

## *5. Home Blood-Glucose Monitors Used by Self-Monitoring Diabetic People*

### 5.1. The Need for Glucose Monitoring in Diabetes Management

In diabetes, a disease of which about 150 million people suffer,[285] the blood and tissue glucose concentrations are not maintained in their normal range by the controlling feedback loops of the body. The blood-glucose concentration of people not afflicted by diabetes is usually in the 70−120 mg/dL, or 4–8 mM range; it is lower when a person is hungry and higher after a meal. In people with diabetes the range is much wider, 30–500 mg/dL or 2–30 mM. Diabetic people perform annually about 6 billion glucose assays, far more than all other assays performed by humanity. While a physician treating a sick person needs to know the results of most of the diagnostic assays (s)he prescribes within hours or days, the diabetic self-monitoring diabetic patient needs to know his or her blood-glucose concentration in less than 20 min in order to avoid life-endangering episodes of hypoglycemia. In extreme cases the rate of decline of the blood-glucose concentration of a type 1 diabetic person is as fast as 4 mM/h.[286]

Type I diabetes affects about 20 million people worldwide and is most frequently diagnosed in children and in young adults. In type 1 diabetes the pancreatic production of insulin, the hormone promoting the uptake of glucose by cells, is impaired. The lives of type I diabetic patients can be sustained only with injections of insulin, which, after meals, lowers the glucose concentration in the blood. In order to maintain their health type I diabetic people need to monitor their blood-glucose concentration 5–6 times a day.

Type II diabetes is much more prevalent than type I diabetes: it afflicts about 5% of the people of the world, most often people who are mature, overweight, and physically inactive. In type II diabetes the insulin produced does not adequately accelerate the uptake of glucose by cells and the insulin-stimulated skeletal-muscle glycogen-synthesis is decreased. The decrease results from reduced insulin-stimulated trans-membrane Glut-4 mediated active glucose transport, caused by intracellular lipid-inhibition of insulin-stimulated insulin-receptor-substrate (IRS)-1 tyrosine phosphorylation, which reduces the IRS-1-associated phosphatidyl inositol-3 kinase activity.[287]

Diabetes has acute and chronic effects. The dramatic, but rare, acute effects, including fainting, coma, and death, result from hypoglycemia. The chronic effects, which are debilitating, result from persistently high glycemia, maintained by many diabetic people in order to avoid the acute effects of hypoglycemia. Persistent maintenance of higher-than-normal blood-glucose concentrations damages the retina, kidneys, nerves, and circulatory system. It is the dominant cause of the reduced longevity of diabetic people, and is the leading cause of blindness among U.S. adults 20–74 years of age, with 12,000–24,000 new cases recorded each year, and also of end-stage renal disease, with about 20,000 cases recorded annually. It accounts for 35% of dialyses and kidney transplants and is the cause of the majority of limb amputations. By controlling his/her glucose concentration within tight limits the diabetic person can avoid the acute hypoglycemia risks and can drastically reduce the likelihood of the devastating complications of diabetes. Tight control is possible only when the diabetic person monitors his or her glucose concentration as often as required by its swings.

### 5.2. Roots of the Electrochemical Glucose Assays Performed by Self-Monitoring Diabetic People

In 1970 Williams, Doig and Korosi demonstrated the first amperometric assay of blood-glucose by a redox couple-mediated, GOx-catalyzed, reaction. They also assayed blood lactate with the FAD-lactate dehydrogenase, FAD-LDH, using $Fe(CN)_6^{3-/4-}$ as redox mediator (reactions 18–20).[98] $Fe(CN)_6^{3-/4-}$ was subsequently very widely used in electrochemical blood-glucose monitoring strips for diabetes management.

$$\text{FAD-LDH} + \text{lactate} \rightarrow \text{FADH}_2\text{-LDH} + \text{pyruvate} \quad (18)$$

$$\text{FADH}_2\text{-LDH} + 2\text{Fe(CN)}_6^{3-} \rightarrow \text{FAD-LDH} + 2\text{Fe(CN)}_6^{4-} + 2\text{H}^+ \quad (19)$$

$$2\text{Fe(CN)}_6^{4-} \rightarrow 2\text{Fe(CN)}_6^{3-} + 2\text{e}^- \quad (20)$$

### 5.3. Gradual Shift from Photonic to Electrochemical Monitoring of Blood-Glucose by Self-Monitoring Diabetic People

The 1970 study of Williams, Doig, and Korosi did not lead to rapid application of amperometry in home blood glucose monitoring. Until 1987 diabetic people assayed their blood-glucose by enzyme-based photonic methods, mostly by measuring change in the light reflectance of a dye-containing stip, resulting in an enzyme-catalyzed glucose oxidation reaction.[286,288,289]

The first electrochemical blood-glucose monitor for self-monitoring diabetic people, which was pen-sized, was disclosed by Higgins, Hill and Plotkin.[290,291] It was launched in 1987 as

ExacTech by Genetics International Inc. of Cambridge, MA. The company subsequently changed its name to MediSense Inc. and was acquired by Abbott Laboratories. In ExacTech, glucose was amperometrically assayed by GOx-catalyzed[292] electroreduction of a ferricinium cation to a ferrocene, which was electrooxidized on a screen printed carbon-paste electrode of a strip.[293,294] The ferrocene/ferrocinium mediator was based on a study of Cass et al., who showed that ferrocene and its derivatives rapidly shuttled electrons from GOx to electrodes.[145]

Today, the majority of the 6 billion annual assays performed by self-monitoring diabetic people are electrochemical. Unlike the photonic assays, the electrochemical assays do not require conversion of an electron current to a photon flux, and reconversion of a photon flux to an electrical current. In addition, the electrochemical strips require smaller blood sample volumes than the photonic strips: all of the presently available strips utilizing sub-microliter blood sample volumes are electrochemical. Also, electrochemical strips can be more easily integrated with automatic and simple fill-detectors, ensuring that an appropriate volume of blood has been applied to the strip.

The home blood-glucose monitors use plastic or paper strips comprising electrochemical cells and contain PQQ-GDH, NAD-GDH, FAD-GDH, or GOx and a redox mediator. The cells and assays differ in the volume of blood they require, in their structure, in their electrode materials, in their redox mediators and in their measurement method. Their measurement method can be amperometry or chronoamperometry or coulometry.[101] An example of an amperometric monitor is the Precision Xtra of Abbott/MediSense. The measurement of FreeStyle, the monitor of TheraSense Inc., now also part of Abbott Laboratories, is microcoulometric. FreeStyle measures the blood-glucose concentration in a blood sample as small as 300 nL, which is painlessly obtained.[295–299]

Considerations in mediator choice include solubility and rate of dissolution, stability in mixtures with proteins, redox potential, availability and cost, and intellectual property rights. Solubility and fast dissolution are important because the fastest assays are now completed in 5 s, the mediator dissolving in the blood sample applied in a second or less.

For example, an older family of home blood-glucose monitors of Roche utilizes enzyme-catalyzed oxidation of all of the glucose in the cell by $Fe(CN)_6^{3+}$, and chronoamperometrically assays the $Fe(CN)_6^{4+}$ produced. An Abbott/MediSense system monitors amperometrically the ferrocene-derivative mediated electrooxidation of glucose. In the coulometric assay of FreeStyle of Abbott/TheraSense, glucose is electrooxidized through an $Os^{2+/3+}$ mediated reaction. Further examples of mediators used in the most recent home blood-glucose monitors are described in Table 1, below.

## 5.4. Practical Considerations in Home Glucose Test Strip Design

The modern commercial electrochemical blood-glucose test strip has a small volume electrochemical cell, utilizes capillary fill, and comprises a stable enzyme and redox mediator. It is accurate (5–10% rms error vs a laboratory standard), fast (5–15 s assay time), and requires a small blood volume (0.3–4.0 $\mu$L). It is produced in high volume (ca. 6 billion total electrochemical strips/year in 2007), at high manufacturing yield and at low cost (5–15 cents/test strip), with a defect rate of less than 0.1%. It can be stored for at least 18 months at room temperature. It fills reproducibly with blood in less than 3 s, typically in 1 s. The commercially available strips also provide a plethora of additional features, including (1) automatic (nonvisual) fill detection, (2) code-free operation, (3) the option to fill the strip with multiple blood aliquots over a period of time, (4) automatic control solution detection, and (5) on-strip hematocrit compensation. This section describes strip designs providing such performance. Figure 2 above shows a sampling of the commercially available electrochemical glucose test-strips.

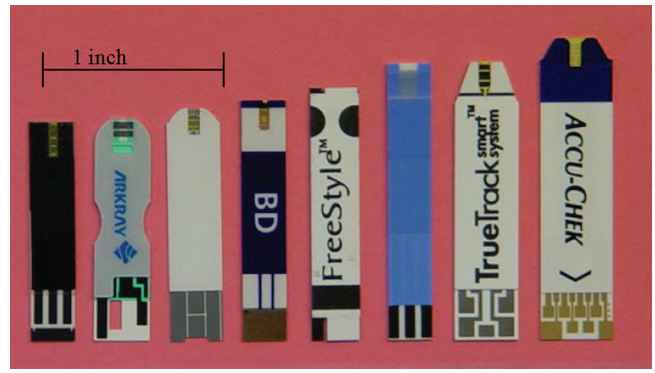



**Figure 2.** A sampling of electrochemical blood-glucose strips with analyzed blood volumes of ca. 1 $\mu$L or less. From left to right, One Touch Ultra, Arkray, Ascensia Contour, BD Test Strip, FreeStyle, Precision Xtra, TrueTrack Smart System, and Accuchek Aviva.

Typical test-strips are about an inch long and a quarter inch wide. This size is dictated by ergonomic cosiderations, as the actual sample chamber (visible at the top for many of the strips) is quite small and occupies only a small fraction of the strip area; all of the shown strips require blood sample volumes of 1 $\mu$L or less. At their bottom, the strips have electrical contact pads, connecting to their respective meters.

The inner workings of a representative strip are pictured in Figure 3, showing six elements common to all electrochemical test-strips. These elements include (1) a plastic substrate material, comprising at least (2) a working electrode and (3) a counter/reference electrode. (The working and counter/reference can also be contained separately on facing plastic substrates, as depicted in Figure 2.) A small volume (ca. 1 $\mu$L) capillary chamber (4) is formed over the plastic substrate(s) and its attached electrodes, often by means of a spacer such as a pressure sensitive adhesive and a cover layer. The strip chemistry (5), consisting of an enzyme, a redox mediator and other components, is distributed (in dry form) within the capillary chamber and generally covers at least the working electrode, and often an entire side of the capillary chamber. Most strips include, in addition, (6) fill detection electrodes, which are vestigial electrodes enabling the meter

**Table 1. Enzyme/Mediator Combinations of Selected Electrochemical Test-Strips**

| strip | enzyme | mediator |
|---|---|---|
| One Touch Ultra | GOx | ferricyanide |
| Arkray | PQQ-GDH | ruthenium hexamine |
| Ascensia Contour | FAD-GDH | ferricyanide |
| BD Test Strip | GOx | ferricyanide |
| FreeStyle | PQQ-GDH | Os complex |
| Precision Xtra | NAD-GDH | phenanthroline quinone |
| TrueTrack Smart System | GOx | ferricyanide |
| Accuchek Aviva | PQQ-GDH | proprietary |

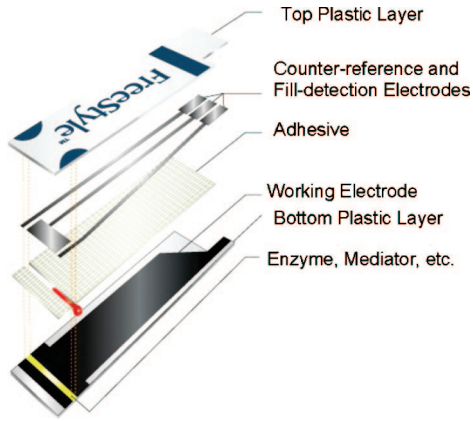



**Figure 3.** Expanded view of an electrochemical blood-glucose monitoring strip used for diabetes management. The capillary chamber is shown partially filled with a droplet of blood. More than a billion of the strips shown are produced annually.

to detect that the strip is sufficiently filled with blood and to initiate, as soon as the cell is filled, the assay. Some strips employ fill detection strategies that do not require additional electrodes.

Ingenious combinations of these six elements allow construction of electrochemical cells precisely measuring the glucose concentration in near- or sub-microliter blood samples. The six common components of strips are described in the following subsections.

### 5.4.1. Plastic Substrates for Home Glucose Test-Strips

The strip body is generally constructed of a thin (ca. 0.005 to 0.015 in.) piece of plastic. It serves as a foundation for the electrodes, which are generally deposited by either screen printing or vapor deposition. The substrate material has a high glass transition temperature, so that high temperature process steps (e.g., drying after application of the strip reagents in liquid form) do not cause distortion of the plastic or its electrodes. Its mechanical strength allows physical handling (e.g., insertion into the glucose meter), yet provides for machinability, such that small sections can be rapidly and accurately cut from a large sheet ("web") of material, during strip production. The most widely used materials are polyesters, such as Melinex and Mylar from DuPont.

### 5.4.2. Working Electrodes for Home Glucose Test-Strips

The working electrode is the portal through which glucose-derived electrons exit the sample and enter the meter. It is most commonly constructed of screen printed carbon ink (a mixture of carbon particles and a polyester binder) or of vapor deposited Au or Pd. Common electrode configurations are illustrated in Figure 4. The working electrode area must be known and constant for the strips to be reproducibly sensitive to glucose. This area is generally defined by the electrode deposition process (i.e., a reproducible area is deposited or scribed), by an insulating dielectric overlayer which masks a reproducible fraction of the working electrode, or by a combination of the two. Generally the active reagents are deposited over the working electrode, but sometimes they are admixed into the conducting materials. The distance between working and counter/reference electrode is minimized, both to reduce the sample volume, and the interelectrode electrolytic resistance.

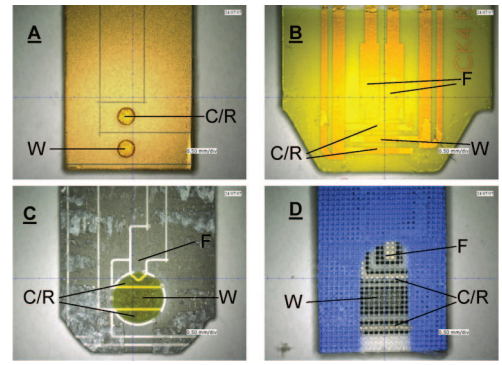



**Figure 4.** Small volume electrochemically blood glucose monitoring strips with their top cover layer removed. W, C/R, and F indicate working, counter/reference, and fill detection electrodes, respectively. (A) BD test strip, with electrodes in recessed wells in an insulating layer. (B) Accuchek Aviva, its fill electrodes doubling as hematocrit-compensation electrodes. (C) Ascensia Contour. (D) Precision Xtra with electrodes covered by a mesh.

### 5.4.3. Counter/Reference Electrodes for Home Glucose Test-Strips

The commercially available electrochemical strips are usually two electrode devices; the counter and reference electrode functions are combined in a single electrode. This counter/reference electrode is the portal through which glucose-derived electrons exit the glucose meter and re-enter the sample. Common configurations are pictured in Figure 4. The counter/reference electrode can be coplanar with the working electrode (in which case it often lies "upstream" of the working electrode) or it can be located on an opposite wall of the capillary cell, such that it "faces" the working electrode. The two often-used types of counter/reference electrodes comprise Ag/AgCl or an inert conductor.

The Ag/AgCl counter/reference electrodes are formed by screen-printing an Ag/AgCl ink, which consists of a mixture of Ag particles and AgCl particles in a polyester binding material (some use a single type of particle with an Ag center and an AgCl periphery). Here glucose-derived electrons react with AgCl to produce Ag, thereby ejecting chloride ions into the sample chamber. Such electrodes are generally designed to have an available Coulombic capacity of reducible AgCl which exceeds by ca. 1 order of magnitude the greatest charge the strip will pass in an actual glucose assay; this available charge is typically a few millicoulombs.

The inert conductor counter/reference electrodes are generally made of the material of the working electrode of a particular strip, such as screen-printed carbon or vapor-deposited Au or Pd. This reduces the cost, because the working and counter/reference electrodes are deposited in the same manufacturing step. The counter/reference electrode functions by reducing part of the excess of oxidized mediator in which it is bathed. Thus, initially, in the dry state, both the working and the counter/reference electrodes are coated with a large excess of oxidized mediator. When glucose-containing blood fills the strip, a small fraction of the excess mediator is reduced via the enzyme-catalyzed reaction with glucose. The working electrode oxidizes the reduced mediator, and the counter-reference reduces additional oxidized mediator from the large available pool. Thus, inert conductor counter/reference electrodes are feasible only in the presence of a large stoichiometric excess of the oxidized mediator over the glucose.

### 5.4.4. Capillary Chamber for Home Glucose Test-Strips

The capillary chamber is the "beaker" of the miniature electrochemical cell. It is formed on at least one broad face by the electrode substrate, and on the other by a cover plate (in facing electrode designs, both surfaces are formed by electrode substrates). A spacer forms the edges. Generally its shape is a rectangular solid, with a width of approximately 1 mm, a length of a few millimeters, and a thickness on the order of 0.1 mm, corresponding to a volume of ca. 1 $\mu$L. It is open at one end to admit the liquid sample, and has an additional opening to allow displaced air to escape as the strip fills. Strip filling can be modeled by the Washburn equation,[300] which can be modified to describe capillary flow between two parallel plates:

$$t = 3\mu x^2/[\sigma \cos(\theta_w)]\, s$$

where $t$ = filling time, $\mu$ = viscosity, $x$ = length (along fill axis), $\sigma$ = liquid surface tension, $\theta_w$ = wetting angle, and $s$ = capillary thickness.

The important result here is that the filling time varies with the square of the chamber length and the inverse of the thickness and the wetting angle. Fill time can, therefore, be decreased greatly by reducing the chamber length. The wetting angle can be decreased by surfactant coating, as described below. In practice, fill times are generally somewhat longer than predicted by the Washburn equation, possibly because of surface discontinuities and formation of the capillary chamber of dissimilar opposite surfaces.

### 5.4.5. Reagents for Home Glucose Test-Strips

The reagents are deposited in dry form over at least the working electrode, but they may cover the entire capillary chamber. In some strips (e.g., Precision Xtra) reagents are mixed directly with the conducting carbon ink, and the mixture is codeposited onto the strip. The reagents include at least an enzyme and a mediator for oxidizing glucose and may further include surfactants to minimize the strip filling time, enzyme stabilizers, and film forming agents, among others. Table 1 lists the enzyme/mediator combinations for some of the available electrochemical strips.

Typical surface-active agents enhancing strip-filling include fluorosurfactants, such as DuPont Zonyl, block copolymers of ethylene oxide and propylene oxide such as BASF Pluronic, or Union Carbide Triton X-100, a nonionic surfactant with a hydrophilic polyethylene oxide group coupled to a hydrocarbon hydrophobic group.

Enzyme stabilizers may include compounds such as monosodium glutamate, trehalose, bovine serum albumin, and buffers (e.g., HEPES, PIPES, etc.) which maintain the enzyme at a favorable pH during storage.

Film forming agents are reputed to improve hematocrit-performance, described below. An example of a film-forming agent is silica, used in One Touch Ultra.

### 5.4.6. Fill Detection in Home Glucose Test-Strips

It is of essence that the strip be completely filled with blood when the electrochemical assay commences. This is obvious for coulometric strips which rely upon a precisely known sample volume to calculate a glucose concentration. It is equally true for amperometric strips, where partial working electrode coverage by blood introduces an error. Formerly, filling was visually confirmed by the user; today it is automated to reduce the likelihood of user error and to make the assay faster. Fill detection is realized in electrochemical strips by:

(1) Positioning the counter/reference electrode downstream (in the sample flow path) from the reference electrode. This condition guarantees that the working electrode is completely covered with blood before the electrochemical circuit is completed by exposure of the counter/reference to blood.

(2) Using identical dual working electrodes, one downstream from the other in the sample flow path. The signal from the two electrodes can then be compared, and the measurement rejected if not sufficiently similar.

(3) Adding another "sensing" electrode downstream from the working electrode. When current is detected at the sensing electrode, the strip is deemed full. There are many variations on this theme; typically the sensing electrode is a small additional working or counter/reference electrode.

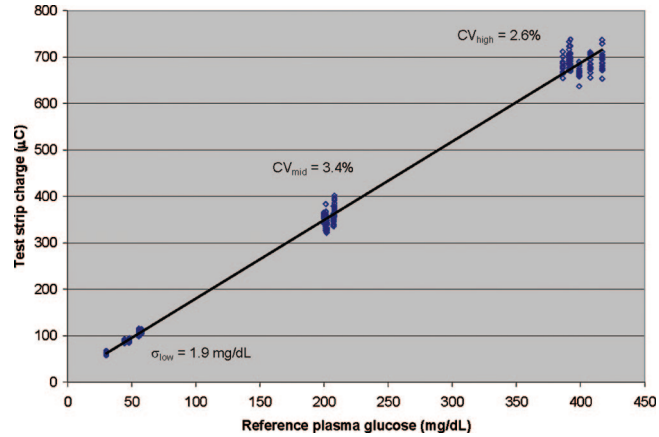



**Figure 5.** Typical lot calibration plot for a coulometric test strip. 298 strips were tested.

## 5.5. Calibration and Characterization of Home Blood-Glucose Test-Strips

The strips pass in quality-contol laboratories a comprehensive battery of tests before their release. Strip lots are first calibrated and are tested to characterize linearity, coefficient of variation (CV), hematocrit dependence, and response to electrochemical interferents, among others. Tests are generally performed with whole blood, but in some cases a formulated blood substitute ("control solution") is used.

### 5.5.1. Calibration of Home Blood-Glucose Test-Strips

Strips are "factory" calibrated by (1) spiking blood samples to low, medium, and high glucose concentrations, then (2) testing these blood samples by both a reference method and by blood-glucose test-strips. Strip response (current or charge) is plotted vs the glucose reference value, as shown in Figure 5, above. The resulting slope and intercept are used to select a strip code for use in the meter, or to accept or reject the strip lot for "codeless" systems. Strips (ca. 300 in Figure 5) are tested from throughout the production lot, which may consist of several hundred thousand strips. "Plasma calibration" is generally used, meaning plasma (prepared by centrifuging the whole blood sample) is tested on the reference device, while whole blood is tested in the strips. Strips so calibrated report an estimated plasma glucose value, even though they are filled with whole blood. This is done to improve agreement with clinical laboratory glucose measurements, typically performed on plasma.

### 5.5.2. Linearity and Coefficient of Variation (CV) of Home Blood-Glucose Test-Strips

Strips should perform accurately over the entire range of clinical interest, generally at least 20–500 mg/dL (ca. 1–30 mM). This performance is gauged by testing multiple (10−20) blood-glucose concentrations spanning the range of interest on both a reference analyzer and the calibrated blood-glucose strips. The CV can be derived from the same data; they are particularly important in gauging a strip's performance, since, unlike nonlinearity, data-scatter is difficult to eliminate by adjusting the calibration algorithm. Commercially available strips generally show a CV of 2–5%. The CV is generally higher at very low glucose concentrations, where strip background (signal in the absence of glucose) makes a significant and variable contribution. The CV is lessened by selecting adjacently produced strips from within a given batch: the within-lot CV is generally larger than the within-vial (the unit of commercial packaging) CV.

### 5.5.3. Hematocrit Dependence of Home Blood-Glucose Test-Strips

Hematocrit, the percentage of blood volume occupied by erythrocytes, has a marked effect on the outcome of the strip-based assay, for a number of reasons. Oxygen from erythrocytes can compete with the redox mediator for glucose-derived electrons in strips when the enzyme used is GOx. Also the viscosity of blood increases with hematocrit, the increase slowing the diffusion of all components and reducing the current in amperometric sensors. In general, there is an inverse relationship between hematocrit and strip-response. The theoretical minimum hematocrit dependence for a plasma calibrated strip is ca. 0.25%/hematocrit unit, based on the 25% solids composition of an erythrocyte. Commercial electrochemical strips generally have a hematocrit-dependence in the range of 0.25–1%/hematocrit unit, although this number can be reduced by hematocrit compensation based on interelectrode impedance measurements. The allowable hematocrit range for electrochemical blood-glucose test-strips varies with strip design; strips with high hematocrit dependence are useful for 25–55% hematocrit, while those with a low innate dependence or hematocrit correction capability can operate over ranges as wide as 0–70% hematocrit.

### 5.5.4. Electrochemical Interferents in Home Blood-Glucose Test-Strips

Electrochemical interferents in blood can cause a false high glucose reading by donating non-glucose-derived electrons. A list of suggested "standard" interferents, developed by the FDA, is shown in Table 2, along with interferent concentrations obtained from the National Center for Clinical Laboratory Standards. (NCCLS Guideline EP7-P, Interference Testing in Clinical Chemistry). Strips are tested at low glucose concentrations, with and without the specified interferents, in order to determine the signal increment due to interferents. From the compounds listed in Table 2, those most likely to electrochemically interfere are ascorbate, acetaminophen, and urate. Strips using a carbon working electrode poised at potentials between about −0.1 V and about 0.2 V versus Ag/AgCl, with fast mediators having redox potentials between about −0.2 V and about 0.1 V versus Ag/AgCl, do not oxidize urate or acetaminophen, the combined blood concentration of which can be as high as 0.6 mM. However, virtually all commercial strips cross-react with ascorbate. Generally, an interference of less than 10% for an interferent concentration at the upper end of the normal physiological range is considered acceptable.

**Table 2. List of Potential Electrochemical Interferents[a]**

| interferent | suggested test level (mg/dL) | ref |
|---|---|---|
| acetaminophen | 20 | 1 |
| salicylic acid | 50 | 1 |
| tetracycline | 4 | 1 |
| dopamine | 13 | 1 |
| ephedrine | 10 | 2 |
| ibuprofen | 40 | 1 |
| L-DOPA | 5 | 3 |
| methyl-DOPA | 2.5 | 1 |
| tolazamide | 100 | 2 |
| Tolbutamide | 100 | 1 |
| ascorbic acid | 3 | 1 |
| bilirubin (unconjugated) | 20 | 1 |
| cholesterol | 500 | 1 |
| creatinine | 30 | 1 |
| triglycerides | 3000 | 1 |
| urate | 20 | 1 |

[a] (1) From NCCLS Document EP7-P; (2) Calculated assuming that the drug, at 10 times the dosage rate, becomes promptly available in 5 L of blood. (3) 10 times the maximum plasma concentration.[301]

### 5.5.5. Additional Testing of Home Blood-Glucose Test-Strips

Testing for dependence on temperature, humidity, and the like is also performed. Of particular interest are "error-stacking" experiments, in which a combination of contingencies designed to elicit particularly egregious errors are tested. For example, combinations of high temperature, low hematocrit, and high ascorbate concentration would provide a particularly large positive error. Results obtained in such tests must be judiciously evaluated with regard to their plausible frequency of occurrence in the real world.

## 5.6. Variables Affecting the Outcome of the Glucose Assays Performed by Self-Monitoring Diabetic People

Because the well being of self-monitoring diabetic people depends on the accuracy of the blood-glucose assays they perform, the outcome of the assays must have minimal dependence on parameters that are not independently monitored and compensated for. Some important sources of error have been discussed above, including hematocrit dependence (5.6.3) and electrochemical interferents (5.6.4). Other sources of error include (1) temperature dependence, (2) skin contamination with glucose or other sugars, (3) improper measurement technique, including miscoding, (4) peritoneal dialysis, and (5) site-to-site variations in glucose concentration.

Because the apparent activation energies can be large, variations with temperature are compensated for, usually by measuring the temperature and applying a correction algorithm. Coulometric systems have an intrinsically lower temperature dependence than amperometric ones, but that does not necessarily translate into a significant difference in operating temperature range (typically about 5–40 °C), because the temperature compensation in amperometric systems is quite accurate.

Skin surface contamination is a significant problem, because a blood droplet can dissolve glucose left on fingertips by food, for example, grapes, in which the concentration

of glucose is particularly high. The skin-contamination problem might have been magnified by the trend to smaller blood sample sizes. As the area/volume ratio of the blood-droplet increases, so does the concentration of a dissolved skin-contaminant. All product labelings request that users wash with soap and water prior to lancing the skin.

Miscoding occurs when the calibration code (assigned to the strip vial during production) is incorrectly entered into the meter. This is addressed by automatic coding "chips" supplied with the strip vial; however, miscoding is still possible if the coding-chip is not used. The best solution is in code-free strips, which rely on either (1) a rigorous selection process during production to limit sensitivity variations, or (2) identifying readable marks on each strip to allow automatic code assignment by the meter.

Peritoneal dialysis involves injection into the peritoneum an iso-osmotic fluid. Icodextrin, a polymer of maltose, is frequently used, resulting in significant elevation of the blood maltose level. Strips made with PQQ-GDH cross-react with maltose, and must not be used by diabetic patients on peritoneal dialysis.[302]

A time-lag may exist between venous glucose levels and those at nonfinger sites, such as the forearm, especially noticeable during periods of rapid change.[303] Lag was observed with an unusual and extreme protocol of Jungheim and Koschinsky for type 1 diabetics: after fasting overnight, the tested people omitted the usual prebreakfast insulin. Instead of breakfast, each patient ingested 75 g of glucose, so that the blood-glucose readings would rise to 300–400 mg/dL; this was followed by the usual mealtime (6−15 units) short-acting dose of insulin. Blood-glucose testing was then performed on the unrubbed forearm, and compared to venous levels. Testing under more realistic conditions suggests that the results for the well-rubbed forearm are accurate, although it is recommended that testing specifically for hypoglycemia be performed on the finger.[304]

## *6. Diabetes Management Based on Frequent or Continuous Amperometric Monitoring of Glucose*

Until the root causes of type I and type II diabetes are eliminated, the life-shortening and quality-of-life damaging consequences of the disease need to be avoided through glucose-monitoring systems. Beyond the single-use strips, frequent and semicontinuous systems, some monitoring the glycemia minute-by-minute are now available. The recently introduced wearable systems utilize subcutaneously implanted, innocuous, nearly painlessly inserted and removed amperometric sensors.

### 6.1. Bedside Glucose-Monitors Measuring the Blood-Glucose Concentration in a By-Stream of Venous Blood

Although there is no evidence to suggest that diabetes is better managed through monitoring the glycemia in the blood than in the interstitial fluid (the fluid between cells) or in the peritoneal fluid, the standard clinical practice of diabetes care has been, and remains, based on monitoring and controlling the blood glycemia. Because it was deemed that diabetes is best managed by monitoring the glycemia of the blood, the earliest intermittent bedside-monitors measured blood-glucose concentrations.[305]

The first bedside system, called the Biostator, was engineered in 1977 by Miles Laboratories of Elkhart, IN. It was a hospital bedside unit, routing a by-stream of blood to an external GOx-based (reaction 7) monitoring unit. Even though only a few hundred units were produced, the Biostator was a core diabetes-research instruments in medical schools and hospitals for more than two decades.[306–309]

At this time Via Medical manufactures a bedside venous blood-glucose monitoring system, the Via Blood-glucose Monitor, sampling a venous by-stream at 5- to 10-min intervals. The sensor of the system is based on reaction 7, with the decline in $O_2$-partial pressure monitored by polarography.[310,311]

### 6.2. Surgeon-Implanted Long-Term Glucose Monitors

Surgeon-implanted, transmitter-containing packages, also based on reaction 7, with footprints larger than 5 $cm^2$, were subcutaneously implanted in animals. Even though some operated for over 100 days, they are not in clinical use. For longevity, their sensors contain a sufficient amount of GOx, stabilized by cross-linking. Their tissue-interface comprises a glucose-flux limiting membrane, ensuring that it is not the enzyme activity, but the glucose permeation-rate through the membrane, that controls the current.[312–315] With an adequate excess of GOx in the membrane-shielded compartment, the usefulness of the implanted sensor-transmitter package depends on the stabilization of the sensitivity. Maintenance of a fixed sensitivity requires fixed glucose-permeability of the membrane, avoidance of adhesion of glucose-metabolizing cells to the membrane, and prevention of encapsulation by glucose-metabolizing tissue. The vascularization of the tissue encapsulating the sensor changes with time. Because change in vascularization perturbs the balance between glucose supply and consumption, the trend has been toward design of sophisticated membranes, many of them modified polyurethanes.[316–318] Those maintained the desired glucose permeation characteristics and either were not encapsulated by tissue, or were overgrown with unchanging well-vascularized tissue, that is not glucose-depleted.

The long-term implants comprise, in addition to their sensor and its associated electronics, a transmitter, consuming most of the power and necessitating a relatively large high energy capacity battery. The battery-life is extended when the distance of to the receiver is shortened, and when the transmissions are less frequent. The trend has, therefore, been to minimize transmission power, accept a short reception distance, and to transmit information about the glucose concentration only at ∼10 min intervals.

### 6.3. Systems with Subcutaneous Ultrafiltration and Microdialysis Fibers and Externally-Worn Sensors

Continuous electrochemical glucose-monitoring systems based on transporting subcutaneous fluid to an external sensor through an implanted sealed-end ultrafiltration fiber,[319–328] or based on forcing the flow of a solution through a microdialysis fiber have been extensively studied.[67,325,329–353] The SCGM1 system of Roche Diagnostics, Mannheim, Germany,[354] and the GlucoDay system of A. Menarini IFR S.r.l, Florence, Italy,[331,345,346,349–352,355] are microdialysis-based. Their microdialysis fibers are implanted in the abdominal adipose-tissue, their fluid flowing to an external amperometric $H_2O_2$-electrooxidizing GOx-utilizing sensor.

The readings of SCGM1 lag by 30 min behind the actual blood-glycemia.[354]

In microdialysis, an isotonic buffer solution is forced to flow through a hollow and microporous fiber. The flowing solution acquires a glucose concentration, which increases with the concentration of glucose in the surrounding adipose tissue. Equilibration with the subcutaneous adipose tissue fluid is only partial, and the glucose concentration difference between the flowing solution and the subcutaneous fluid depends on the flow rate and on the extent of fouling of the fiber by proteins and adhering cells. The useful life of both the ultrafiltration and the microdialysis-based systems is determined primarily by fouling and bacterial contamination of their fibers and other compartments and only secondarily by inflammation, that is, glucose-consuming macrophages, near the fiber. The growth of any organism in any exterior or interior compartment of the ultafiltration or microdialysis system, or of any cell on the ultafiltration or microdialysis fiber, lowers the apparent concentration of glucose and makes the relating of the subcutaneous glucose concentration to the measured current particularly problematic under hypoglycemic conditions.

In ultrafiltration fiber-based systems the implanted end of the fiber is sealed, and the distal end is connected to the externally worn sensor compartment, which is connected to an evacuated cylinder, into which the fluid is sucked. The evacuated container is replaced daily. In microdialysis, one fiber terminus resides in the isotonic buffer solution and the other end is connected to the sensor compartment, which is, in turn, connected to an evacuated container. The equilibration and flow-rates, and the volume of the sensor compartment, determine the lag of the measured glucose concentration behind that in the monitored tissue.[340–342,345,347,349,356] A novel microdialyzer adds a constant glucose concentration to the perfusate and operates in a pulsatile flow mode, eliminating the need for calibration.[343]

## 6.4. Reverse-Iontophoretic Systems

The GlucoWatch G2 Biographer (GW2B) sold, then discontinued, by Animas Technologies LLC, now part of Johnson & Johnson, iontophoretically transports fluid across the skin to an externally worn glucose monitor.[357] Its sensor consisted of a pair of Pt-graphite working electrodes, each surrounded by an Ag/AgCl iontophoretic electrode, contacting a GOx-containing hydrogel pad, which, in turn, contacted the skin. A current of 0.3 mA was applied for 3 min through a Ag/AgCl electrode pair, resulting in the ionophoretic transport of glucose into the GOx-containing hydrogel pad, where the glucose was air-oxidized according to reaction 7. The iontophoresys-driving current was then switched off, and a 0.42V vs Ag/AgCl potential is applied to the working electrode for 7 min to completely electrooxidize the $H_2O_2$ produced in reaction 7.[357] The time required for an assay was ~10 min. The concentration of glucose in the iontophoretically derived fluid was well below that in the subcutaneous interstitial fluid, but the glucose concentrations in the two fluids were related. The GW2B measured glucose concentrations and detected trends, identifying hypoglycemic and hyperglycemic events.[358] A study[359] of 89 pediatric patients, who wore 174 GW2Bs, showed a mean relative absolute difference vs laboratory serum values of 22%, similar to the then available semicontinuous subcutaneously implanted monitors.[360] Utility was limited by iontophoresis-induced skin irritation and missed readings, paricularly in periods of perspiration.[361]

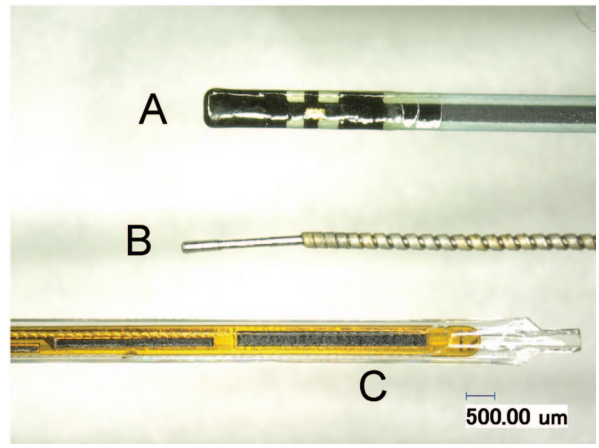



**Figure 6.** Commercially available transcutaneous sensors. (A) FreeStyle Navigator; (B) Dexcom STS; (C) Guardian RT.

## 6.5. Subcutaneously Inserted User-Replaced Miniature Amperometric Sensors

Transcutaneous amperometric sensor based systems are at this time the dominantly used continuous glucose monitors. A thin, sub-1 mm diameter, flexible sensor, having a working electrode with an immobilized enzyme (usually GOx) and an AgCl/Ag counter or counter-reference electrode is inserted under the skin. The electrooxidation of glucose is mediated by either $O_2$ (6.5.1), or by an immobilized redox mediator (6.5.2). A glucose flux-limiting membrane (6.5.3) overlays at least the working electrode of the sensor. Although it limits the glucose electrooxidation currents to the nA range, it provides for linear glucose-response over the range of clinical interest, and also provides a biocompatible tissue interface. Representative transcutaneous sensors are shown in Figure 6.

The sensor is inserted into the subcutaneous fat, to a depth of 5–10 mm, usually by a hollow, retractable sharp. Its leads are connected to a small on-skin potentiostat, typically equipped with a wireless transmitter. A suitable break-in period of 1–10 h is then observed, both to equilibrate the sensor with its tissue-environment and to normalize the insertion-wound perturbed site. The sensor is then calibrated *in vivo*, usually by assaying a blood droplet using a strip, and continuous glucose readings then commence, typically every 1–5 min.

### *6.5.1. Subcutaneously Inserted User-Replaced Miniature Sensors Based on GOx Catalyzed Generation of $H_2O_2$ and Its Electrooxidation*

These systems, reviewed earlier by Wilson and Hu,[362] and by Wilson and Gifford[363] contain GOx immobilized on the working electrode, and do not contain an immobilized mediator. Their chemical and electrochemical reactions are those of reactions sections 6.5.1–6.5.3. The $H_2O_2$ produced by reactions 1 and 2 is electrooxidized at the working electrode poised near 0.6 V vs Ag/AgCl, and the $H_2O_2$-electrooxidation current is monitored. Systems available to diabetic people include Medtronic's Guardian REAL-Time System and DexCom's STS continuous glucose monitor. The sensor of the Guardian REAL-Time System consists of a flexible plastic substrate, less than 1 mm in width, with

coplanar working, reference, and counter electrodes formed by a lithographic process.[364] GOx is immobilized on the working electrode, the flux limiting membrane is coated over the electrode-bearing substrate, and the entire assembly is enclosed in a plastic sleeve equipped with a hole over the working electrode. The system monitors glucose concentrations through the 40–400 mg/dL range and its subcutaneous sensor has a recommended usage time of 3 days between replacements.

The Dexcom STS is a two electrode system, with an Ag/AgCl counter-reference electrode. It comprises an insulated Pt wire, around which a chlorided Ag-wire is coiled. The insulation is stripped off a segment of the Pt, and GOx is immobilized on the wire to form the working electrode. The flux-limiting membrane is deposited around both the working and the counter electrodes. Its monitor reports glucose concentrations in the 40–400 mg/dL range. The sensor is also replaced by the user every 3 days.[366] An improved version of this sensor, with a 7 day wear-time called *Seven*, has been recently approved by the FDA and is available.

### 6.5.2. Implanted Amperometric Glucose Sensors Built on the Wiring of Glucose Oxidase

Glucose is directly electrooxidized, in the absence of $O_2$ and without generation of $H_2O_2$, at electrodes coated with electrocatalysts made by electrically wiring GOx through an electron conducting redox hydrogel, as described in section 2.5. The first wiring-based amperometric glucose-monitor for diabetes management is FreeStyle Navigator of Thera-Sense, now part of Abbott Diabetes Care. Its disposable 5-day wired glucose oxidase glucose sensor is implanted and replaced by the patient with minimal pain and the presence of the miniature sensor under the skin is not felt by the wearer. The surface of the sensor is designed to lessen cell adhesion.[365–367] The sensor consists of a narrow (0.6 mm wide) plastic substrate on which carbon-working, Ag/AgCl-reference, and carbon-counter electrodes in a stacked geometry are screen printed. The active wired enzyme sensing layer covers a small fraction, only about 0.1 $mm^2$, of the working electrode, and all electrodes are overlaid by a flux-limiting membrane. The sensor resides at about 5 mm depth in the subcutaneous adipose tissue and monitors glucose concentrations over the range 20–500 mg/dL. It is replaced by the user, practically painlessly, after 5 days of use.

### 6.5.3. Flux-Limiting Membranes for Transcutaneous Amperometric Sensors

Membranes on transcutaneous sensors serve two purposes: (1) limiting the glucose flux to the sensing element, and (2) providing a biocompatible interface between the sensor and the body. These functions and their realization are described below.

The current increases linearly with the glucose concentration if all of the arriving glucose molecules are electrooxidized. If the glucose oxidation process is not fast enough to allow the electrooxidation of all of the glucose influx, the electrooxidized fraction of glucose molecules decreases as the glucose concentration increases and a nonlinear current/concentration dependence results. The membranes for the devices described here reduce glucose flux by a factor of approximately 10–100×, enabling the glucose oxidation process to "keep up" with the incoming glucose molecules over the range of roughly 20–500 mg/dL.

Another benefit of encapsulating the sensing layer is the enhancement of its stability. Aging-related decline in sensitivity is avoided by use of the membrane, because even if part of the activity of the glucose electrooxidation catalyst is lost, the residual limiting rate of glucose electrooxidation is still high enough to ensure that all the arriving glucose molecules are electrooxidized. Use of membranes that are excessively resistive to glucose transport is nevertheless avoided, so as to avoid a delay greater than about 3 min between the measured and the true glucose concentration. A longer delay would introduce an excessive measurement error when the glucose concentration rises or declines rapidly.

The glucose transport-limiting membrane defines an apparent Michaelis constant of the sensor, $k_M^{app}$. Because the current is controlled by the rate of permeation of glucose through the membrane, the temperature-dependence of the glucose-electrooxidation current is defined by the activation energy for glucose permeation.

The flux of glucose to the sensor membrane surface depends linearly on its solution concentration as long as the viscosity is invariant. This is, however, not the case if the sensor is progressively fouled by a glucose-flux-blocking deposit or if glucose-consuming cells grow on or in the proximity of the sensor. To reduce fouling and adhesion of glucose-consuming cells, the membrane should also be bioinert. Although most membranes were made of polymers, the use of cubic-phase lyotropic liquid crystalline membranes having reproducible and uniform thicknesses and easy to reproduce glucose-permeabilities has also been proposed.[368] The patent literature discloses proprietary bioinert membrane examples, such as poly(vinylpyridine)-derived hydrogels[369] and swellable polyurethanes.[370]

For oxygen-mediated peroxide-measuring sensors, the membranes must be sufficiently permselective for $O_2$ over glucose for the rate of reaction 2 not to be controlled by the $O_2$-concentration, which can be in the subcutaneous fluid of a diabetic person 300 times smaller than the glucose concentration (0.1 mM versus 30 mM).[371–376] This restriction does not exist for wired glucose sensors, which do not require oxygen for the glucose-measuring reaction. The latter are therefore compatible with more hydrophilic membrane materials, which do not exhibit as great a preference for oxygen permeation as do some more hydrophobic materials.

### 6.5.4. Calibration of Transcutaneous Amperometric Sensors

When the function relating the signal $s$ and the concentration $c$ is both known and unchanged after implantation, or if it changes predictably after implantation, then a precalibrated sensor does not require recalibration after its implantation. If the function relating $s$ and $c$ changes upon or after implantation, then the sensor must be recalibrated *in vivo*. This last condition generally obtains and "factory calibration" of transcutaneous sensors is not presently offered, for reasons including the following: (1) their more complicated manufacturing process results in greater within-lot coefficient of variation, typically on the order of 10%, as tested *in vitro*, as compared to the 3–5% variation of single-use test-strips; (2) the *in vivo* coefficients of variation being typically larger than the *in vitro* variations, because of the variability of the subcutaneous environment; and (3) sensor output drift and tissue renormalization following insertion-wounding. The presently necessary *in vivo* calibration is performed by implanting and applying an operating potential to the

subcutaneous sensor, allowing a suitable equilibration period of 1–10 h, testing the capillary blood glucose concentration using a glucose test strip, and calculating the ratio of the resultant glucose concentration to the transcutaneous sensor current to obtain an operating sensitivity. Generally, an intercept value (transcutaneous sensor current at zero glucose concentration) must be assumed. The value of the intercept is smaller for wired glucose sensors operating at very low potentials (ca. 40 mV vs Ag/AgCl), than it is for hydrogen-peroxide electrooxidizing sensors, which operate at ca. 500 mV vs Ag/AgCl. Note that *in vivo calibration* is inherently less accurate than glucose test-strip factory-calibration, because a consumer glucose meter is usually used for reference in the *in vivo* calibration, and an accurate desktop clinical glucose-analyzer is used for reference in test-strip factory-calibration.

Strategies for improving the accuracy of the *in vivo* calibrations[377,378] include (1) averaging simultaneous duplicate capillary measurements; (2) rejecting calibrations at extreme glucose concentrations and during periods of extremely rapid glucose concentration change; (3) preassigning a calibration code to the transcutaneous sensor so as to limit the range of sensitivities allowed by the *in vivo* calibration process; (4) incorporating a capillary glucose meter in the transcutaneous sensor hardware to eliminate errors resulting from incorrect user transcription of the capillary glucose value, and also to eliminate errors associated with systematic variations between the various commercial glucose meters; and (5) extending the postimplantation waiting period to eliminate erroneous early sensitivities stemming from the insertion wound associated trauma.

The *in vivo* calibration process must be repeated during the implantation to correct for possible sensitivity changes. The frequency of these repeat calibrations differs from manufacturer to manufacturer. The FreeStyle Navigator observes a 10 h equilibration period, followed by four *in vivo* calibrations over the following 5 days. The Dexcom STS requires a 1 h equilibration followed by twice daily calibrations over the ensuing 3-day wear, as does the Guardian RT. All systems allow the user to input additional calibration values, if calibration accuracy is deemed to be uncertain.

Calibration-free operation is presently an important research and development objective, both to increase user satisfaction, and to eliminate errors in the user-performed calibration process. Its development will require more reproducible transcutaneous sensor manufacturing processes, as well as better understanding of the factors causing sensitivity-variation *in vivo*.

### 6.5.5. The Relationship between the Glucose Concentrations in Blood and in the Subcutaneous Interstitial Fluid

When the blood-glucose concentration neither rises nor declines, then the glucose concentrations in the blood and in the subcutaneous fluid are similar, or, if not exactly similar, they are related through a proportionality constant. During periods in which the blood-glucose concentration rises or drops, the two concentrations differ.

The relationship of the difference between a sensor-measured glucose concentration and the true glucose concentration is often expressed by a grid proposed by Clarke et al. The zones in this grid are lettered A to E. In zone A the measurement correctly reflects the actual glucose concentration, whereas in zone E the error of the measurement is such that if a clinical decision were based on it, the patient might be harmed. A difference smaller than about 15% between the actual and the measured glucose concentrations is considered as unlikely to lead to an incorrect clinical decision. Greater differences must, however, be avoided, particularly at or near hypoglycemia. The source of the difference between the actual and the measured glucose concentrations can be sensor-related or physiological. A true physiological difference exists when the glucose concentration rises or falls, the difference being a function of the rate at which the glucose concentration changes; the difference is approximately proportional to the slope, $dc/dt$.[379–382]

If, in an animal experiment, the blood-glucose concentration is forced to decline at 6 mg/dL min$^{-1}$, the magnitude of the transient blood-subcutaneous fluid glucose concentration difference can approach the measured concentration. Schmidtke et al.[380] found that when insulin (0.5 U/kg) was intravenously injected in the rat, the subcutaneous concentration was transiently higher, by as much as 84%, than the blood-glucose concentration, the difference reaching its maximum 25 ± 7 min after the injection. Even though the difference was large, they were able to model it mathematically. The modeling provided an algorithm for translating the measured subcutaneous glucose concentration to the blood-glucose concentration at the instant of the subcutaneous measurement. Because the translation required knowledge of $dc/dt$, its quality depended on the frequency of sampling and thus on the response-time, $\tau_{90\%}$, of the sensor. For sensors with $\tau_{90\%}$ of 2 min the average difference between the blood concentrations and the subcutaneous concentrations during the 40-min period after the injection of insulin was reduced from 84% to 29%.[381,382]

Under normal conditions, after intramuscular insulin injection in a type I brittle diabetic chimpanzee, the fastest decline in a series of five experiments was 1.8 mg/(dL min).[386] Although the resulting difference between the blood and the subcutaneous glucose concentrations was readily measurable, the magnitude of the difference was unlikely to lead to an improper clinical decision by the standards of Clarke et al. In these experiments the subcutaneously implanted miniature wired glucose oxidase electrode was operated in conjunction with an on-the-skin ECG Ag/AgCl electrode. The chimpanzee was unconstrained and was trained to wear a small electronic monitor on her wrist and to present her heel for obtaining capillary blood samples. In five sets of measurements with five sensors, averaging 5 h each, 82 capillary blood samples were assayed, their concentrations ranging from 35 to 400 mg/dL. After one-point calibrations were performed at $t = 1$ h, the rms error in the correlation between the sensor-measured glucose concentration and that in capillary blood was 17.2%, only 4.9% above the intrinsic 12.3% rms error of the reference strip-monitor. The capillary blood and the subcutaneous glucose concentrations were statistically indistinguishable when the rate of change was <1 mg/(dL min). However, when the rate of decline exceeded 1.8 mg/(dL min) after insulin injection, the subcutaneous glucose concentration was transiently higher.

After considerable research, consensus has emerged on the intrinsic averaged time-lag in humans being about 7 min between the subcutaneous interstitial fluid glucose concentration and the venous blood concentration. Boyne et al. examined 14 patients wearing the Medtronic CGMS,

and found a mean lag relative to venous blood of about 7 min.[384] A study using the Glucowatch Biographer[385] found a mean delay of 17.2 min, of which 13.5 min were attributed to instrumental delay. Similarly, a study with 30 subjects wearing FreeStyle Navigator found a mean delay of 8 min, of which 3 min could be attributed to sensor response time.[378] A subsequent study[386] with the same sensor, in 58 subjects, found a mean lag of 9.5 ± 4.6 min, again inclusive of a 3-min sensor response time. Standard deviation of this lag was larger between insertions (4.9 min) than within insertions (2.6 min), suggesting real intrasubject and/or intrasite differences.

The continuous glucose monitors Guardian, DexCom, FreeStyle Navigator, and Glucoday had, respectively, during euglycemia, mean absolute relative differences of 15.2%, 21.2%, 15.3%, and 15.6%; during hypoglycemia, 16.1%, 21.5%, 10.3%, and 17.5%. In Clarke error-grid analyses, during euglycemia, respectively, 98.9%, 98.3%, 98.6%, and 98.7% of the data points were in zones A + B. Because of frequent loss of sensitivity, the DexCom did not provide sufficient data during hypoglycemia when respectively 84.4%, 97.0%, and 96.2% of the data points of Guardian, FreeStyle Navigator, and GlucoDay were in Clarke zones A + B.[387]

## 6.6. Research Aimed at Integrating a Miniature Power-Source in a 5-Day Patient-Replaced Subcutaneously Implanted Glycemic Status Monitoring and Transmitting Package[388,389]

All presently used batteries contain reactive, corrosive, or toxic components and require cases, usually made of steel. As a battery is miniaturized, the required case dominates its size. Hence, the smallest manufactured batteries are of about 50 $mm^3$, much larger than the integrated circuits and sensors of functional implantable amperometric glucose sensors for diabetes management.

### 6.6.1. The Potentially Implantable Miniature Zn/AgCl Cell[391]

The presently manufactured small batteries, usually zinc−air, zinc−silver oxide, or lithium−manganese dioxide cells, are difficult to miniaturize because they require a steel case to contain the caustic $KOH_{aqu}$ of Zn batteries or lithium, which reacts rapidly with water. Because the volume fraction of the case increases upon miniaturization, size reduction, though feasible, becomes impractical. The battery could be miniaturized if the anode and the cathode, as well as their reaction products, would be safe enough for implantation in the subcutaneous interstitial fluid so the fluid would serve as the electrolyte, and both the electrolytic solution and case would be obviated.

In a step toward such an implantable battery, the $KOH_{aqu}$ electrolyte and the steel-case of a Zn-anode battery were eliminated, opening a route to practical implantable micro-batteries. Specifically, the out-diffusing $Zn^{2+}$, generated in the anode reaction $Zn \rightarrow Zn^{2+} + 2e^-$, was precipitated on the surface of the Nafion coated Zn anodes by phosphate, to form nonporous lamellae of hopeite-phase $Zn_3(PO_4)_2 \cdot 0.4H_2O$. Surprisingly, the hopeite-phase $Zn_3(PO_4)_2 \cdot 0.4H_2O$ was a $Zn^{2+}$-cation conducting solid electrolyte. Nonporous inorganic films are usually impermeable to gases, including $O_2$. Hence, they block or reduce non-Faradaic corrosion. However, because they are rarely ionic conductors, the corrosion-protected Zn-anodes can usually not be discharged and are not useful in batteries. The $O_2$-associated corrosion of the Zn-anode was, nevertheless, reduced in a pH 7.4 physiological (0.15 M NaCl, 20 mM phosphate) buffer solution by growth of a hopeite-phase $Zn_3(PO_4)_2 \cdot 0.4\ H_2O$ $Zn^{2+}$-conductive film on the anode. Because the film prevented permeation of $O_2$ to the electroactive metallic Zn surface, the Zn was efficiently utilized, even when the anode's surface to volume ratio was high and its rate of discharge was slow. For example, the zinc utilization efficiency of 120 $\mu$m diameter Zn fiber anodes, discharged over 3-weeks, was 86%. Growth of the nonporous hopeite lamellae and high anode utilization efficiency required precoating with a polyanion, like Nafion, and the addition of a halide like NaCl. The anodes were discharged with little polarization, even when they were overgrown by 100 $\mu$m thick hopeite films. At a current density of 0.13 mA $cm^{-2}$ the excess polarization of the half-discharged, 100 $\mu$m hopeite-overgrown anodes was less than 50 mV. At 0.26 mA $cm^{-1}$, the polarization of the half-discharged anodes exceeded their initial polarization only by about 110 mV. The ionic conductance of the hopeite lamellae was $>2 \times 10^{-3}$ S.

The zinc anodes, as well as the pH 7.3 physiological buffer electrolyte of the foreseen cells, are harmless enough to be considered for implantation. One cathode explored was the implantable Ag/AgCl cathode, used in FreeStyle Navigator described in section 6.5.2. Because the Ag/AgCl cathode is already used in the body, it is expected that the Ag/AgCl cathode should be safe to implant.

Tests of the Zn(Nafion)-Ag/AgCl cell showed that the nonporous, $Zn^{2+}$ conducting, hopeite-phase $Zn_3(PO_4)_2 \cdot 4H_2O$ grows on the Nafion coated zinc anode also when the pH 7.4, 0.15 M NaCl, 20 mM phosphate buffer is replaced by serum and that the zinc utilization efficiency remains near 60% when the cell is discharged in serum at a 2-week rate at 1 V. It is projected that the cell will operate in the less fouling subcutaneous interstitial fluid at least as well as it operated in serum.

### 6.6.2. The Potentially Implantable Miniature Zn−$O_2$ Cell

The energy density of the implantable cell of 6.6.1 would be increased about 10-fold if instead of AgCl/Ag an air-cathode would be used. At physiological pH the cathode could be wired bilirubin oxidase,[391–395] overcoated with a bioinert $O_2$ permeable, proton transporting polymer or hydrogel, or with a cubic phase lyotropic liquid crystal, which is permeable to $O_2$, water, and ions but excludes part of the many damaging serum-constituents, including intermediates of urate oxidation.[396] The polarization of the wired bilirubin-oxidase $O_2$ electroreduction catalyst is about 0.3 V less than that of platinum at equal current density, making it superior for use in physiologal pH $O_2$-cathodes.[391]

### 6.6.3. The Potentially Implantable Miniature Glucose-$O_2$ Biofuel Cell

The energy capacity could be further increased by a membrane-less and case-less implanted cell utilizing the glucose and the dissolved $O_2$ of the subcutaneous interstitial (intercellular) fluid. Because in the glucose-$O_2$ biofuel neither the cathodic reactant, $O_2$, nor the anodic reactant, glucose, is cell-contained, but instead diffuse to the electrodes from the subcutaneous fluid, the cell is the smallest. Its laboratory version consists merely of two 7 $\mu$m diameter, 2 cm long carbon fibers, one the wired glucose oxidase coated anode

and the other the wired bilirubin oxidase coated cathode. The cell operates optimally at 0.6 V, where its power density is about 4.8 $\mu$W mm$^{-2}$ of carbon fiber area.[395,397,398]

In its weeklong operation at 37 °C such a cell generated ~1 J of electrical energy. The charge passed was 2 C, exceeding about a hundredfold the 0.016 C charge that would have been passed in the discharge of a zinc fiber anode of similar dimension (7-$\mu$m diameter, 2 cm long) at 100% utilization efficiency. Unlike other fuel and biofuel cells the enzyme-wiring based glucose-$O_2$ cell is simple and potentially inexpensive, because its anode and cathode compartments need not be separated by a membrane.

The need for the membrane is avoided in biofuel cells when their anodic and cathodic electrocatalysts are wired enzymes. Unlike the platinum alloy electrocatalysts of other fuel cells, the wired-enzyme electrocatalysts are so selective for their substrates, that neither the crossover of glucose to the cathode compartment, nor the crossover of $O_2$ to the anode compartment, harms the cell. Furthermore, unlike the platinum alloy-utilizing cathodes, which are rapidly poisoned by carbon-containing oxidation intermediates, the wired bilirubin oxidase cathode is not poisoned by glucose nor by its oxidation product, gluconolactone. Because the cell does not have diffusional anodic or cathodic redox mediators, which would short it by the mediator being reduced at the anode and oxidized at the cathode, there is no need for the separation of the compartments. In absence of a membrane, the biofuel cell consists merely of the two wired enzyme coated electrodes, which would be overcoated in the actually implantable version with a bioinert hydrogels, permeable to glucose, $O_2$, and ions.[389]

The glucose-$O_2$ biofuel cell operates at this time for about a week in pH 7.3 physiological buffer, containing 0.14 M NaCl and 20 mM phosphate, for about a day in the living grape, the sap of which is particularly rich in glucose,[395] but only for hours in serum.

## 7. Concluding Remarks

Electrochemical glucose monitoring has contributed massively to improving the lives of diabetic people. As the prevalence of diabetes is increasing worldwide and as curing of the two types of diabetes remains elusive, humanity is likely to further benefit from advances in the electrochemical monitoring of glycemia.

Following the single-use strips, which now painlessly and accurately monitor glucose concentrations in 300 nL samples of blood, increasingly advanced, continuous glucose monitors are being introduced. Their already small subcutaneous sensors will be further miniaturized, and their 5–7 day operating lives will be extended. Through increasingly accurate and continuous measurement of the first and second derivatives, the diabetic user will be adequately forewarned if and when corrective action is needed to maintain a healthier narrower, closer to normal, glycemic range. The pain of blood glucose monitoring has been eliminated and the constant worry of diabetic people is now being eliminated through progress in bioelectrochemistry.

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