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Electrochemistry in Diabetes Management

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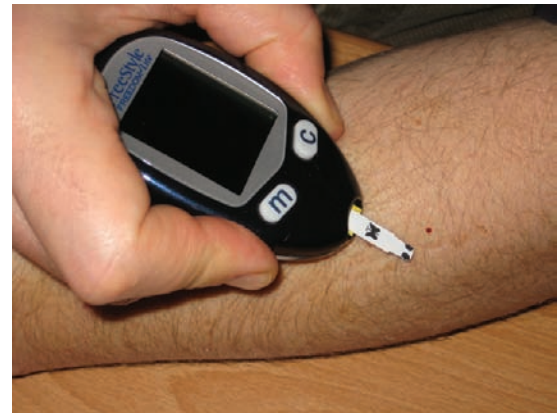

Diabetes devastates lives and burdens society. Hypoglycemic (low glucose) episodes cause blackouts, and severe ones are life-threatening. Periods of hyperglycemia (high glucose) cause circulatory disease, stroke, amputations, blindness, kidney failure and nerve degeneration. In this Account, we describe the founding of TheraSense, now a major part of Abbott Diabetes Care, and the development of two products that have improved the lives of people with diabetes. The first, a virtually painless microcoulometer (300 nL volume), the FreeStyle blood glucose monitoring system, was approved by the FDA and became available in 2000. In 2009, this system was used in more than one billion blood assays. The second, the enzyme-wiring based, subcutaneously-implanted FreeStyle Navigator continuous glucose monitoring system, was approved by the FDA and became available in the United States in 2008.

The strips of the FreeStyle blood glucose monitoring system comprise a printed parallel plate coulometer, with a 50 μm gap between two facing printed electrodes, a carbon electrode and a Ag/AgCl electrode. The volume of blood between the facing plates is accurately controlled. The glucose is electrooxidized through catalysis by a glucose dehydrogenase (GDH) and an $Os^{2+/3+}$ redox mediator, which is reduced by the glucose-reduced enzyme and is electrooxidized on the carbon electrode. Initially the system used pyrroloquinoline quinone (PQQ)-dependent GDH but now uses flavin adenine dinucleotide (FAD)-dependent GDH. Because the facing electrodes are separated by such a small distance, shuttling of electrons by the redox couple could interfere with the coulometric assay. However, the $Os^{2+/3+}$ redox mediator is selected to have a substantially negative formal potential, between 0.0 and -0.2 V, versus that of the facing Ag/AgCl electrode. This makes the flow of a shuttling current between the two electrodes virtually impossible because the oxidized Os^{3+} complex cannot be appreciably reduced at the more positively poised Ag/AgCl electrode.

The FreeStyle Navigator continuous glucose monitoring system uses a subcutaneously implanted miniature plastic sensor connected to a transmitter to measure glycemia amperometrically and sends the information to a PDA-like device every minute. The sensor consists of a narrow (0.6 mm wide) plastic substrate on which carbon-working, Ag/AgCl reference, and carbon counter electrodes are printed in a stacked geometry. The active wired enzyme sensing layer covers only about 0.1 mm^2 of the working electrode and is overlaid by a flux-limiting membrane. It resides at about 5 mm depth in the subcutaneous adipose tissue and monitors glucose concentrations over the range 20$-$500 mg/dL. Its core component, a miniature, disposable, amperometric glucose sensor, has an electrooxidation catalyst made from a crosslinked adduct of glucose oxidase (GOx) and a GOx wiring redox hydrogel containing a polymer-bound $Os^{2+/3+}$ complex. Because of the selectivity of the catalyst for glucose, very little current flows in the absence of glucose. That feature, either alone or in combination with other features of the sensor, facilitates the one-point calibration of the system. The sensor is implanted subcutaneously and replaced by the patient after 5 days use with minimal pain. The wearer does not feel its presence under the skin.

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Introduction and Prologue

Unlike two 2008 comprehensive reviews of electrochemical glucose monitors,[1,2] one of which we coauthored, this Account is personal. It tells the stories of people at the University of Texas, TheraSense, and Abbott Diabetes Care who improved the lives of diabetic people.

Diabetes devastates lives and burdens society. Hypoglycemic (low glucose) episodes cause blackouts and when severe, they are life-threatening. Periods of hyperglycemia (high glucose) cause circulatory disease, stroke, amputations, blindness, kidney failure, and nerve degeneration. The societal burden of diabetes is also heavy. More than 17 million people in the US suffer from diabetes. In 2007, their per capita direct cost of health care exceeded that of nondiabetic people by as much as $10,000. The disease hits poor people particularly hard: in 2002 the blood sugar concentration of 49% of the indigent diabetic Medicaid recipients was out-of-control, while the blood sugar concentration of only 23% of the better-off diabetic Medicare recipients was out-of-control.

In 1994, based on research at the University of Texas in Austin (UT), Adam and his son Ephraim Heller cofounded E. Heller and Company at UT's Technology Incubator mostly to maintain the health of diabetic people with less pain, complications, and anxiety. Their initial objective was to develop an enzyme-wiring based continuous glucose monitor (CGM). Research prototypes of the monitor were built by Adam and his colleagues at the University of Texas at Austin (UT). The cost of introducing the wired-enzyme CGM system was, however, over $100 million, beyond the means of the small company. Part of the required funds later became available through the much less costly development of another successful product, a blood glucose monitor requiring only 300 nL blood, which was the basis for a $120 million initial public offering of TheraSense stock in the fall of 2001. These funds allowed TheraSense to develop and carry out the first clinical trials of its wired-enzyme CGM system. In 2004, TheraSense was acquired by Abbott Laboratories for $1.26 billion, and the company became a part of Abbott Diabetes Care (ADC). ADC completed the development and clinical testing of the wired-enzyme CGM and named it the *FreeStyle Navigator* continuous glucose monitoring system. It was introduced in the European Community in 2007 and in the US in 2008. The *FreeStyle Navigator* system reduces the frequency of both hypoglycemic periods, in which life-threatening blackouts occur, and hyperglycemic periods, which can result in macrovascular and microvascular complications.

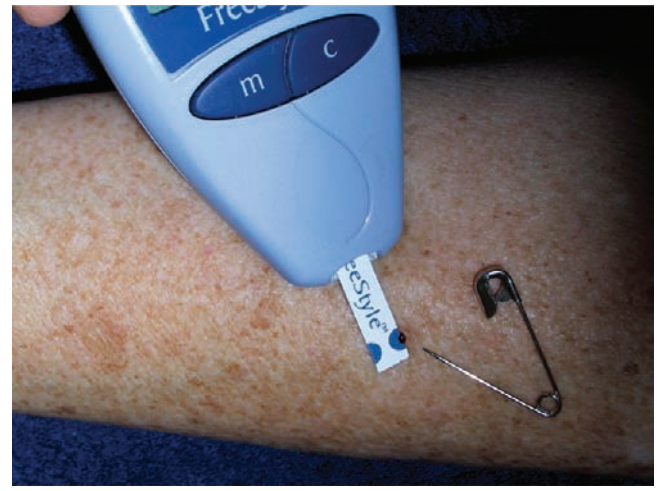


FIGURE 1. The *FreeStyle* microcoulometric strip and meter and a blood sample. The photograph is of the arm of Adam's wife, Ilana, taken in 2000.

Painless Glucose Monitoring in 300 nL of Blood

Over 10 billion glucose assays are performed annually by diabetic people. Their number vastly exceeds the combined number of all other chemical and biochemical analyses performed by humanity. In 1995, Ephraim moved E. Heller & Co. to Alameda, CA, and later renamed its component developing diabetes-related products TheraSense. Because the still small company did not have the required funds to develop its wired-enzyme CGM, Ephraim refocused TheraSense on painless blood glucose monitoring. This was based on using extremely small (submicroliter) blood samples, through the *FreeStyle* system, the first practically painless glucose monitor for diabetes management (Figure 1).[3] The blood sample that it required was reduced to 300 nL, $^1/_8{}^{th}$ of the blood volume of an average mosquito meal. In 1996, the blood volumes required for home glucose assays were generally of 3−5 μL, with some as large as 10 μL. These were obtained by lancing the fingertips, which was painful but could supply the required substantial blood volumes. Experimenting on his forearm, Ephraim recognized that upon lancing the skin of his arm with the same lancets that were used to obtain the large blood volumes from fingertips, much smaller, generally submicroliter, blood samples were obtained. The tiny samples were obtained, unlike those obtained from the fingertips, painlessly. Consequently, Ephraim asked us whether we could design a glucose test strip for submicroliter blood samples.

Photometric, light absorption, and reflection-based home blood glucose monitors preceded the electrochemical monitors. These systems work through the enzymatic transfer of glucose-derived electrons to light-absorbing dye molecules,

followed by a reflectance measurement. Unlike the electrochemical monitors, the photometric systems require exclusion of red blood cells as well as an assayed area of at least a few square millimeters in order to perform the reflectance measurement. For these reasons, photometric glucose test strips have not followed electrochemical test strips into the submicroliter realm, and today they are used in a declining minority of the glucose assays. The revolutionary shift from photometric to electrochemical glucose self-monitoring began in 1987, when Genetics International, which was renamed MediSense in 1988 and was acquired by Abbott Laboratories in 1996, introduced the first electrochemical monitor, ExacTech.[4] It consisted of a strip and a pen, with the glucose assayed amperometrically in the strip and the pen comprising a potentiostat and a display. The electrooxidation of glucose was enzyme and redox mediator catalyzed, the mediator being a ferrocene-derivative originating at the University of Oxford; part of the strip development was contracted to Cranfield University. The group published in 1984 a milestone paper describing its amperometric glucose monitor.[5] Recognizing the advantage of electrochemical assays, the other manufacturers of photometric strips began the development of electrochemical assays, all of which involved enzyme-catalyzed oxidation of glucose, reduction of an oxidized redox mediator by the glucose-reduced enzyme, and electrooxidation of the resulting reduced mediator. The $K_3Fe(CN)_6^{3-/4-}$ redox couple was often used as mediator; commonly used enzymes included glucose oxidase, reduced nicotinamide adenine dinucleotide (NADH)-glucose dehydrogenase, and pyrroloquinoline quinone (PQQ)-glucose dehydrogenase. In all pre-*FreeStyle* system assays, the glucose concentration was obtained by monitoring the steady-state or decaying current associated with reduced mediator electrooxidation.[1,2] The use of a coulometric glucose assay was proposed by Ben in 1996. The reasons for the preference of coulometry over the then practiced amperometric and chronoamperometric methods were twofold. First, in the coulometric assay, the measured electrical signal varied more linearly with the glucose concentration than it did in amperometry under the constraints of our 300 nL cell volume. Second, the outcome of coulometry, unlike that of amperometry, was only modestly affected by kinetic variables like temperature, blood viscosity, glucose electrooxidation-catalyzing enzyme activity, or the preferential electrooxidation of reducing blood constituents like vitamin C or urate. The small interelectrode separation, coupled with the fast redox mediator/enzyme chemistry, made it possible to complete the near-exhaustive coulometric oxidation of glucose in a very short time. In the *FreeStyle* system, we used parallel facing working and counter electrodes separated by about 50 μm for two reasons: (1) facing electrodes at least halved the required electrode area relative to the then standard coplanar designs, leading to a smaller required blood volume, and (2) for facing electrodes the interelectrode resistance and mean electrode separation were much smaller than for coplanar electrodes, allowing a faster assay.

In order to use closely spaced facing electrodes, we had to overcome the hurdle of redox shuttling. Most of the coplanar strips that were in use at the time relied on the rapid enzyme-mediated oxidation of glucose by a large molar excess (relative to glucose) of initially oxidized redox mediator, followed by electrooxidation of any reduced mediator produced by reaction with glucose. This electrooxidation was effected by application of a substantial (ca. 400−500 mV) potential difference between the coplanar working and counter electrodes. Under the constraints of high potential difference and large molar excess of redox mediator, the use of a facing electrode design would have led to an enormous background current, caused by the repeated oxidation and reduction of mediator at the working and counter electrodes. The underlying phenomenon is called redox shuttling, the term pointing to the tendency of the redox mediator to "shuttle" back and forth between the closely spaced electrodes.

In order to avoid the spurious charge that could have been added by the shuttling of a diffusing redox mediator between the reducing and the oxidizing electrodes, we built the initial version of the microcoulometric cell with a "wired" PQQ-glucose dehydrogenase anode.[6] This prevented electron shuttling, because the redox mediator was immobilized on the working electrode and could not "shuttle" to the counter electrode. However, because at the time we did not have as yet the very fast enzyme wires that we now have, the assay would have required about 45 s. This was incompatible with the diabetic user-demanded 15 s or shorter assay. To shorten the assay, Ben proposed replacing this immobilized mediator by a soluble redox mediator with an oxidation potential substantially negative of that of the Ag/AgCl counter electrode but positive of the potential of the glucose-reduced enzyme. Such a mediator was completely oxidized at the working electrode by application of a potential of zero (0 mV) between working and counter electrodes. Under the condition of zero applied potential between the facing electrodes, redox shuttling was thermodynamically impossible, and the electrochemical cell was rendered galvanic.

At the working (printed carbon) electrode, the half-cell reaction is the enzyme- and mediator-catalyzed oxidation of glucose:

$$\text{glucose} \rightarrow \text{gluconolactone} + 2H^+ + 2e^- \quad (1)$$

The corresponding reductive half-cell reaction is the reduction of AgCl:

$$AgCl + e^- \rightarrow Ag + Cl^- \quad (2)$$

Thus the net reaction is

$$\text{glucose} + 2AgCl \rightarrow \text{gluconolactone} + 2Ag + 2H^+ + 2Cl^- \quad (3)$$

Reaction 3 is spontaneous, and the strip is essentially a very small glucose/AgCl battery with a Coulombic capacity proportional to the glucose content. Reaction 3 continues until the glucose within the strip is mostly oxidized, in contrast to amperometric devices, which typically either consume only a small fraction of the glucose or, if reacting all the glucose with an oxidized redox mediator, consume only a small fraction of the reduced redox mediator produced. Note that the strip (Figure 4) is a two electrode (working and counter/reference) device, even though there are four separate electrodes. At three electrodes, the counter/reference and the two fill detecting electrodes of Figure 4, the redox processes are the same. This subdivision of current is performed in order to confirm, by separately measuring the current through the fill electrodes, that the cell of the strip is filled with blood. The cell does not have a reference electrode.

Because reaction 3 is spontaneous, in the early versions of the monitor the potential difference between the working and counter/reference electrode was actually maintained at 0 V. In later versions, the applied potential (working vs Ag/AgCl counter/reference) was increased to +100 mV. Redox shuttling is still avoided at +100 mV, when the rapidly electrooxidized/electroreduced $Os^{2+/3+}$ redox mediator is selected to have a formal potential substantially negative (between −0.0 and 0.1 V) versus that of the facing Ag/AgCl electrode. This makes the flow of a shuttling current between the two electrodes virtually impossible, since the oxidized Os^{3+} complex cannot be appreciably reduced by the more positively poised Ag/AgCl. The first such easily oxidized redox mediator, shown in Figure 2, was designed by Ben based on results of Adam and his colleagues in Austin on the redox potential of a related polymer.[7] Specifically, Ben replaced the N-vinyl function of a polymer, having a redox potential of −105 mV vs Ag/AgCl,[7] with an N-methyl function, without substantially altering the potential. The monomeric *N*-methylimidazole comprising the mediator was synthesized by Caroline Anderson at TheraSense.

Use of the diffusing, low potential redox mediator of Figure 2 reduced the time of the assay to less than 15 s, and the

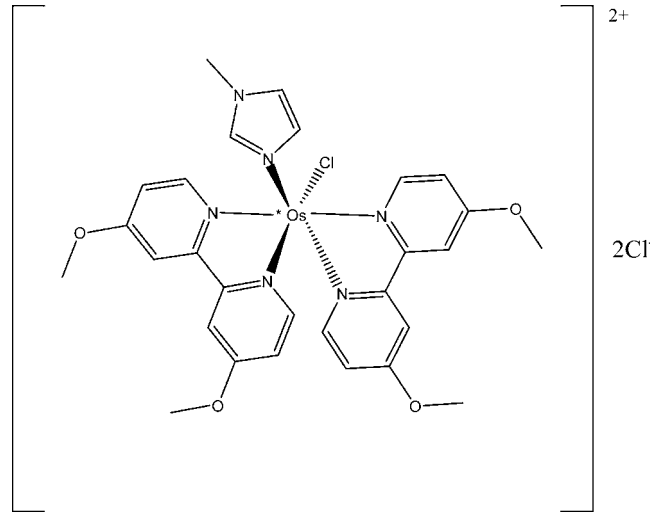


FIGURE 2. The original *FreeStyle* mediator tailored to have a redox potential positive enough to oxidize glucose-reduced PQQ-glucose dehydrogenase yet negative versus the redox potential of the Ag/AgCl electrode. It enabled operation of the thin-film testing strips in a galvanic mode, without redox shuttling. The oxidized member of the redox couple is shown.

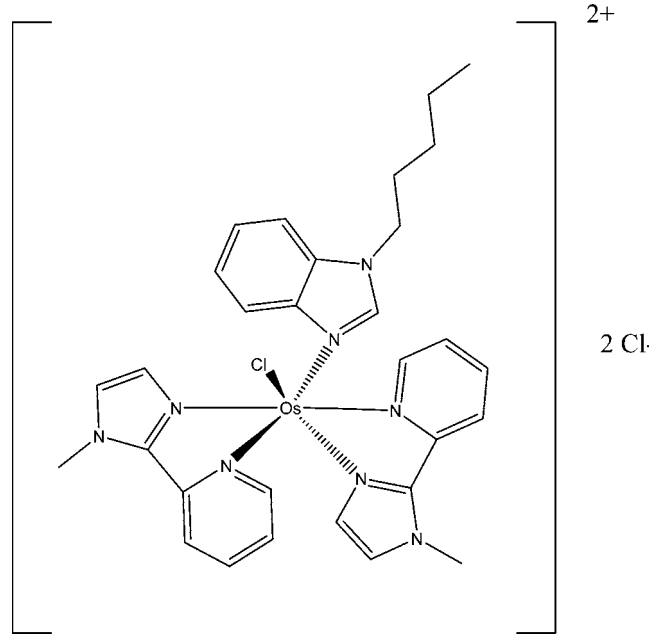


FIGURE 3. The 2nd generation redox mediator for FreeStyle test strips. The oxidized member of the couple is shown.

mediator, along with freely diffusing PQQ−glucose dehydrogenase (GDH) enzyme, were used in the first commercial version of the test strip, released in 2000. Many of the experiments confirming the viability of this design were performed by Rajesh Krishnan. The strip chemistry continued to evolve, however, and was further improved upon the introduction of the redox mediator of Figure 3. This fast redox mediator, with a potential of −125 mV vs Ag/AgCl, was designed by our TheraSense colleague Fei Mao, who later

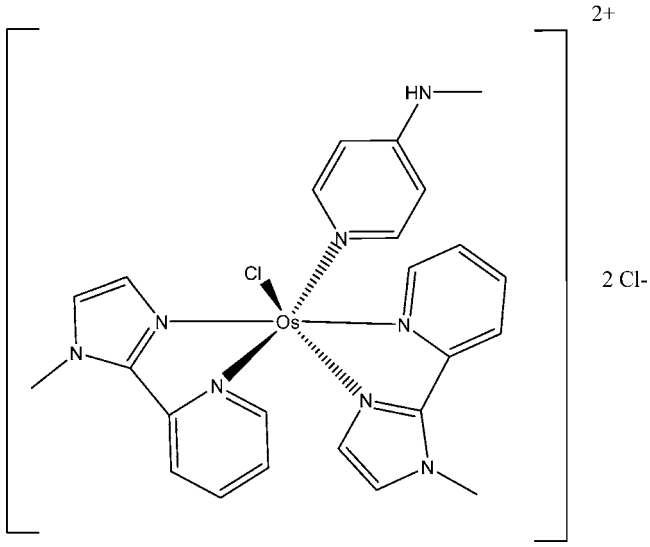


FIGURE 4. The current redox mediator for *FreeStyle* test strips, designed for a high current density with the FAD-GDH enzyme. The oxidized member of the couple is shown.

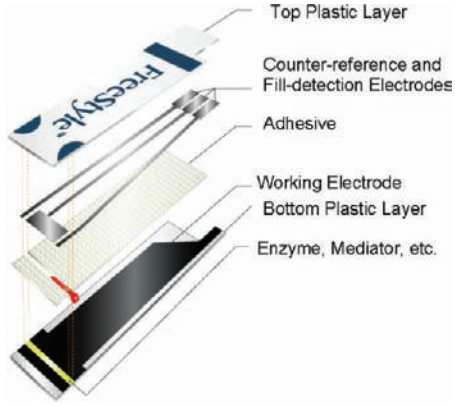


FIGURE 5. The *FreeStyle* test strip containing the 300 nL microcoulometric cell.

founded and became President of Biotium of Hayward, CA. The mediator, along with changes in the electronics and algorithm, allowed a further 3-fold reduction of the test time, to 5 s.

A change in mediator was made when the PQQ-GDH was changed to flavin adenine dinucleotide (FAD)-GDH. The structure of the current redox mediator, with a potential of −160 mV vs Ag/AgCl, is shown in Figure 4. This mediator also allows a fast 5 s testing time, while providing a higher average current in conjunction with FAD-GDH, than does the mediator of Figure 3.

The volume-dependent coulometric assay required the reproducible mass manufacture of the 50 μm gap thin-layer electrochemical cell of Figure 5 with a coefficient of variation of 2−3% or less. The gap was maintained initially by using a spacer with cut windows, then by applying a surprisingly reproducible film of a pressure-sensitive adhesive. We credit

FIGURE 6. The *FreeStyle Navigator* system, the "wired" glucose oxidase-based, subcutaneously implanted continuous blood glucose monitor. The display shows a glycemia of 109 mg/dL; the directional arrow shows that the glycemia is increasing at a moderate rate. The sensor is implanted under the transmitter shown on the arm; the information is transmitted to and displayed by the hand-held display.

our manufacturing engineer, Phil Plante, one of the first people Ephraim hired at TheraSense, with their mass-production. He was ably assisted by Joe Vivolo, who expanded and improved the production.

The painless coulometric monitor, fondly named *Colossus* in internal TheraSense documents, was released as the *FreeStyle* blood glucose monitoring system. The *FreeStyle* system was approved by the FDA and became available to diabetic people worldwide in 2000.[3] Having produced billions of the 300 nL coulometric cells of Figure 4, we showed that submicroliter fluidic devices can be reproducibly mass-manufactured.

Continuous Glucose Monitoring with the *FreeStyle Navigator* System

Abbott Diabetes Care (ADC) completed the development and clinical testing of the wired-enzyme CGM of TheraSense and named it the *FreeStyle Navigator* continuous glucose monitoring system. It was introduced it in the European Community in 2007 and in the US in 2008. The *FreeStyle Navigator* system (Figure 6) has been shown, in clinical trials, as well as in independent studies,[8] to reduce the frequency of both hypoglycemic periods, in which life-threatening blackouts occur, and hyperglycemic periods, which can result in macrovascular and microvascular complications. Earlier studies[9] have shown that reducing hyperglycemic excursions results in a decrease of microvascular and macrovascular complications. The *FreeStyle Navigator* system improves glucose control by (1)

providing real-time glucose data, (2) issuing alarms when the glucose level exceeds high and low preset values, and (3) predicting future values and issuing predictive alarms before the limiting thresholds have been surpassed. The monitor is based on the electrical wiring of glucose oxidase (GOx), which underlies the direct conversion of the concentration-dependent glucose flux to an electrical current.

Research on enzyme wiring began around 1987 at Bell Laboratories, where Adam and Yinon Degani decorated glucose oxidase with redox centers that rapidly exchanged electrons. The centers, covalently bound to the protein, formed electron tunneling paths.[10] The wiring of GOx enabled direct transduction of the concentration-dependent fluxes of glucose to electrical currents and provided a new interface between electronics, medicine, and biology. In their first experiment, Yinon and Adam decorated glucose oxidase (GOx) with ferrocene/ferricinium redox centers,[10] so as to create phonon-assisted electron tunneling paths between the $FADH_2$ centers of GOx and gold or carbon electrodes. The paths enabled the electrooxidation of glucose without use of a diffusional, and therefore leached, redox shuttle between the $FADH_2$ of GOx to the electrodes. To form these paths, amines of inner protein domains of GOx were reacted with a carbodiimide-activated ferrocene/ferricinium carboxylate ester to form ferrocene carboxamides. Decoration of the partly unfolded protein with the ferrocene/ferricinium redox centers turned the electronically insulating protein into an electron conductor and after renaturing, the buried GOx $FADH_2$ redox centers transferred electrons to electrodes via the protein-bound ferrocene/ferricinium centers. Because the FAD of the conducting GOx was reduced by glucose to $FADH_2$, glucose was directly electrooxidized and its electro-oxidation was observed as a current.[10]

In 1987, Bell Laboratories was still the world's prime center of research on electronic materials and devices. It was, however, not the best place to develop a new electronics−medical−biological interface. For this reason, Adam relinquished his position as head of Bell Laboratories' Electronic Materials Research Department in 1988, and moved to UT. Through his first year at UT, Adam continued to work part time at Bell Laboratories, where he and Yinon showed that the $FADH_2$ redox centers of GOx can be electrically "wired" to an electrode not only by decorating the protein with fast redox centers but also by precipitating on electrodes thin water-insoluble films of the electrostatic adduct of GOx, a polyanion at neutral pH and a polycationic copolymer of vinylferrocene.[11,12] At UT, Brian Gregg and Adam then introduced the use of electron-conducting hydrogels, which had no leachable components, for the wiring of enzymes: they covalently bound GOx within the hydrogels, electrically wiring the enzyme's redox centers.[13−15] The redox hydrogels were cross-linked polymers, usually containing osmium polypyridine-based redox centers tethered to the cross-linked water-soluble polymer backbones, the tethers preventing their leaching. The gels were reversibly electroreduced and electrooxidized, their redox centers transferring electrons to and accepting electrons from their closest neighbors.

When enzymes like GOx were incorporated in the mostly $Os^{2+/3+}$-complex-based redox hydrogels, the enzyme and the redox polymer, the entropy of mixing of which was small, phase-separated on the nanoscale. To prevent phase-separation, an electrostatic adduct of GOx, a polyanion at the physiological pH of 7.3, and an excess of the polycationic redox polymer was formed, then cross-linked. The GOx became electrically wired by the redox-conducting hydrogel, that is, when the enzyme's FAD centers were glucose-reduced to $FADH_2$, they reduced the Os^{3+} centers of the polymer to Os^{2+}. When the wired GOx was immobilized by cross-linking on an electrode poised at a sufficiently oxidizing potential, the Os^{2+} was electrooxidized. This made the wired GOx an electrocatalyst for the oxidation of glucose.

The enzyme-wiring redox hydrogels, which had no leachable components, had four unique properties: first, they constituted electron-conducting aqueous phases, the only ones in existence;[16,17] second, they electrically "wired" multilayers of GOx molecules; third, they made unnecessary the orientation of GOx so as to minimize the distance between the $FADH_2$ redox centers and the electrodes; and fourth, they were highly permeable to glucose, to gluconolactone, the product of GOx, and to electrolytes. They provided three-dimensional electrocatalysts having no leachable redox mediators[14,18,19] and glucose electrooxidation current densities of $>10^{-3}$ A cm^{-2}.[20] Consequently, with currents as small as 10^{-10} A routinely measured, Mike Pishko, now Professor and Head of the Chemical Engineering Department of Texas A&M University at College Station, and Adam were able to form 7 μm diameter glucose electrooxidizing microelectrodes.[21] With these in hand, the UT team miniaturized CGM electrodes to a diameter of 250 μm.[22,23] The UT CGM electrodes were implanted and tested in the following order: first in Adam, then in Ephraim, then in Adam's wife Ilana, then in rats infused with glucose and with insulin,[23−26] then in a diabetic chimpanzee,[27] and ultimately in diabetic patients. The UT studies formed the basis for the CGM of TheraSense, which became Abbott Diabetes Care's *FreeStyle Navigator* system (Figure 6). After multicenter clinical trials, the *FreeStyle Navigator* system

FIGURE 7. Electron conduction in redox hydrogels results in electron transfer between reduced and oxidized redox centers tethered to the backbone of polymers.

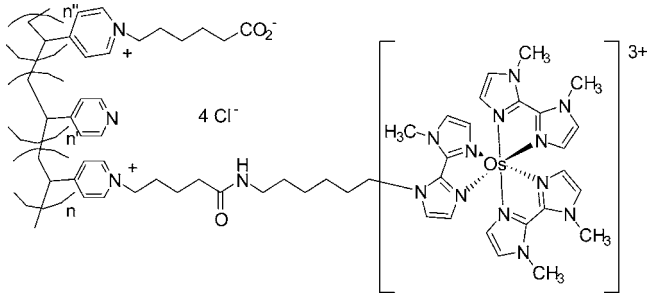


FIGURE 8. A "wire" of glucose oxidase, allowing the electrooxidation of glucose at 40 mV vs Ag/AgCl at a current density of 1.3 mA cm^{-2}. The 5.8 $\times$ 10^{-6} cm^2 s^{-1} apparent electron diffusion coefficient in the hydrogel formed of the cross-linked polymer approaches the diffusion coefficient of ions, like Cl^-, in water. $n = 11$, $n' = 85$ and $n'' = 4$; the mer distribution is about random.

obtained regulatory approvals and was released in Europe in 2007 and in the United States in 2008.

Electrons are transported in the GOx-wiring gel through collisions between segmentally mobile Os^{2+} and Os^{3+} redox centers tethered to the polymer's backbone[16] (Figure 7). A state-of-the art GOx-wiring polymer, synthesized by Fei Mao, is shown in Figure 8.[20,28] The polymer of Figure 8 is polycationic at physiological pH and forms a phase-separation-preventing electrostatic adduct with GOx, which is a polyanion at the pH of the subcutaneous fluid.

Because in the polymer of Figure 8 the redox centers do not change their structure upon reduction and oxidation, their Marcus reorganization energy is small and the centers relay electrons rapidly.[19] When the tethers between the backbones and the redox centers were made long and flexible, the amplitudes of the bound redox centers were large. As a result, electrons diffuse in the hydrogels about as rapidly as their charge-balancing ions diffuse in water. The apparent electron diffusion coefficient of the redox hydrogel formed by cross-linking and hydrating the polymer of Figure 8 is 6 $\times$ 10^{-6} cm^2 s^{-1}, similar to that of freely diffusing Os-based monomeric redox couples.

Highly selective glucose electrooxidation is important not only for accuracy of the CGM but also for proper operation and one-point calibration of the implanted sensors.[22] Proper operation requires that the user be able to test a drawn blood sample, for example, with the *FreeStyle* blood glucose system, and compare the result with that displayed by the CGM, for example, by the *FreeStyle Navigator* system. Also determination of the sensitivity, that is, the dependence of the current generated on the glucose concentration, of an implanted amperometric sensor relies on knowledge of the currents at different glucose concentrations. For a two-point calibration, the diabetic user would have had to measure the current at two glucose concentrations, for example, would have had to wait for rise or decline of the glycemia. Such calibration would have been, to say the least, deterring the use of the system. If, however, the current varied practically exclusively with the glucose concentration, then the sensor could be calibrated without waiting for the glucose concentration to change, for example, by performing a single painless *FreeStyle* blood glucose assay.

The *FreeStyle Navigator* working electrode selectively oxidizes glucose at the relatively mild potential of 0.04 V vs Ag/AgCl. This property contributed to successful one-point calibration of the implanted *FreeStyle Navigator* sensors and also to successful one-point validation of their readings.[22,26] In the earliest version of the *FreeStyle Navigator* system, additional selectivity was achieved by a layer that chemically oxidized the influx of oxidizable interferants. The layer contained lactate oxidase and horseradish peroxidase. Lactate in the tested fluid was O_2-oxidized to pyruvate, while the O_2 was reduced to H_2O_2. The H_2O_2 oxidized the horseradish peroxidase, which in turn oxidized the oxidizable interferants.[29] A disadvantage of this method of eliminating the reactants that could have interfered with instantaneous validation and one point calibration was that it required dissolved O_2. For this reason, the reactive interferant elimination was replaced by down-shifting the redox potentials of the GOx-wiring redox hydrogels to potentials where the interferant-electrooxidation-associated currents were small,[7,30,31] then negligible.[28]

Acceptably steady glucose sensitivity for >5 days was provided, along with a resolution of 0.2 mM across the 2−30

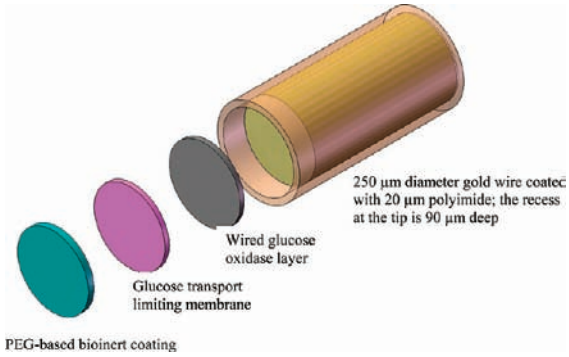


FIGURE 9. Structure of a 1998 version of the continuous glucose monitoring subcutaneously implanted "wired" GOx electrodes: gray, "wired" enzyme sensing layer; pink, glucose transport controlling membrane; aqua, biocompatible poly(ethylene oxide) film. The three layers are deposited in the 90 μm deep recess formed by etching the gold of the tip of a polyimide insulated gold wire. The diameter of the wire is 250 μm; the thickness of the polyimide layer is 20 μm. Courtesy of Christopher Thomas, Abbott Diabetes Care.

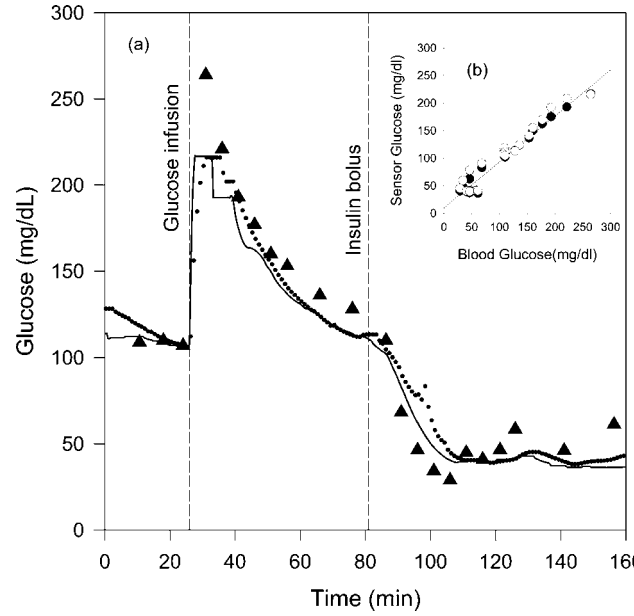


FIGURE 10. Establishment of the similarity of the blood and the interstitial fluid glucose concentrations. The glycemia of the rat following venous glucose and insulin infusions. Glucose concentration (▲) in withdrawn blood samples, (···) in the vein, measured by the wired glucose oxidase sensor, (—) in the subcutaneous interstitial fluid, measured by the wired glucose oxidase sensor. The insert shows the correlation of the blood and the subcutaneous interstitial fluid glucose concentrations.

mM range relevant to the management of diabetes, by a tailored glucose-flux-limiting membrane. When a membrane reduces the influx of glucose sufficiently, the current is determined by the glucose flux through the membrane, not by the less stable activity of the electrocatalyst. Figure 9 shows a schematic diagram of the implanted working electrodes that Ephraim and Adam wore and that Adam's UT team later implanted in rats. As counter–reference electrodes, commercially available EKG Ag/AgCl and NiO_x/NiO_y electrodes were used. The implanted electrodes, of 0.29 mm diameter, were formed in polyimide insulated gold wires, the tips of which were etched by Sten Eric Lindquist to form a 0.09 mm deep cavity. The gold tips were coated by Elisabeth Csoeregi with three successive layers of wired GOx, of glucose flux-reducing poly(ethylene glycol) diglycidyl ether-cross-linked poly(*N*-vinylimidazole), and of a bioinert, cross-linked, poly(ethylene glycol), such as its diacrylate.[23,32] The bioinert layer, reducing fouling and immune reaction, leading to accumulation of glucose-consuming and oxidant generating leukocytes, was designed with Christopher Quinn, now Director of Research and Technology of Akzo-Nobel Surface Chemistry in Brewster, New York, and Jeff Hubbell,[33,34] now Director of the Laboratory for Regenerative Medicine and Pharmacobiology of EPFL Lausanne.

Experiments around 1995–2000 laid to rest a misconception of the existence of a massive difference between the blood and subcutaneous interstitial fluid glucose concentrations. Although the two differed during rapid rise or decline of glycemia, they were, most of the time, similar (Figures 10 and 11).[25,27]

The production versions of the *FreeStyle Navigator* system incorporated design changes to simplify manufacture and enhance performance. First, the sensor itself was changed from a hollow tube design to a coplanar printed circuit in which working, reference, and counter electrodes were incorporated onto a single polyester substrate, as shown in Figure 12. In this design, the three screen printed electrodes were stacked one atop each other, separated by intervening dielectric insulator, to minimize the size of the implanted portion of the sensor.

In addition, the multilayer coating of Figure 9 was replaced by a single membrane made of a polymer cross-linked with a poly(glycidyl ether). It combines the dual functions of limiting glucose flux and providing a bioinert interface. This polymer is shown in Figure 13. It is applied by reproducible processes, yielding sensors with predictable sensitivity.

Clinical trial results utilizing the membrane of Figure 13 and a sensor structure similar to that of Figure 12 were published in 2003.[35] The *FreeStyle Navigator* system monitors and stores the subcutaneous glucose concentration at 1 min intervals. It has a transmitter mounted on and electrically contacting the subcutaneous sensor and a hand-held receiver–computer display (Figure 6). It has user-set hypoglycemic and hyperglycemic alarms. Its miniature subcutaneous sensor, a

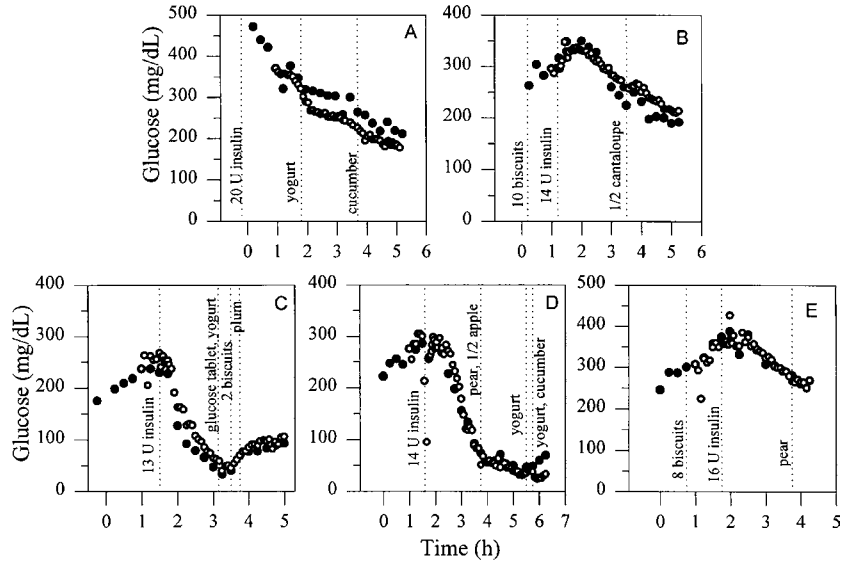


FIGURE 11. Comparison of results of blood glucose assays (●) and the readings of the subcutaneously implanted monitor implanted in a type 1 brittle diabetic chimpanzee (○).

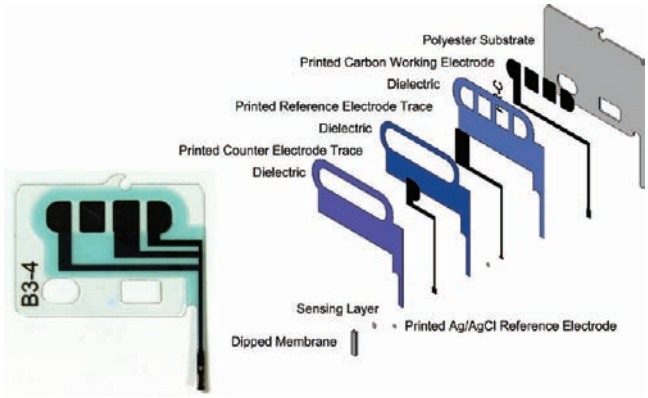
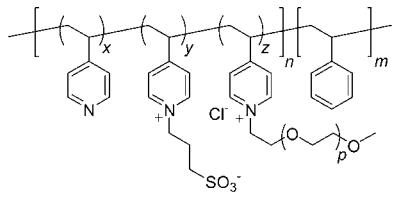


FIGURE 12. Production version of the *FreeStyle Navigator* sensor, with diagram showing the multiple stacked component layers. Courtesy of Christopher Thomas, Abbott Diabetes Care.



Where x =0.85, y=0.1, z=0.05, n=9, m=1, and p= ca. 10.

FIGURE 13. The current *FreeStyle Navigator* outer membrane polymer. It provides simultaneously, after its cross-linking with a poly(glycidyl ether), the glucose-limiting flux barrier and the bioinert interface.

plastic sensor, is replaced by the patient every 5 days. By measuring both the glucose concentration and its rate of change, the *FreeStyle Navigator* system warns of both actual and impending hypoglycemia[36] or hyperglycemia, allowing the user to take corrective action, that is, eating or drinking to raise the glycemia or administering insulin to lower it.

Epilogue and Conclusion

On September 29, 2008, Adam was awarded the U.S. National Medal of Technology and Innovation by President Bush in a White House ceremony for "fundamental contributions to electrochemistry and bioelectrochemistry, and the subsequent application of those fundamentals in the development of technological products that improved the quality of life across the globe, most notably in the area of human health and well-being." Ben and two E. Heller & Co., TheraSense, and Abbott colleagues were present: TheraSense founder Ephraim Heller, who identified the need for and defined the blood volume required for the painless monitoring of glucose and made it the objective of TheraSense, and Tim Goodnow, Vice President R&D of TheraSense and now Divisional Vice President, Technical Operations of ADC, who managed the complex development and engineering of the continuous glucose monitor, involving chemistry, chemical engineering, materials science and engineering, electrical engineering, microelectronics, software engineering, mechanical engineering, process engineering, and endocrinology.

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BIOGRAPHICAL INFORMATION

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 Ph.D. in Organic Chemistry in 1961 from Ernst David Bergman at the Hebrew University in Jerusalem. After postdoctoral work at UC Berkeley (1962−1963) and at Bell Laboratories (1963−1964), he joined At GTE Laboratories

(1964−1975), where he built the first Nd^{3+} liquid laser and, with J. J. Auborn, the worldwide manufactured and used Li/$SOCl_2$ battery. At Bell Laboratories (1975−1988), he designed the first >10% efficient electrochemical solar cells and the first >10% efficient hydrogen-generating solar-powered photoelectrodes. At Bell Laboratories, he also headed the Electronic Materials Research Department (1977−1988), where part of the high density chip interconnection technology underlying the miniaturization of portable electronic devices has been developed. 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 2008 removes the worry of diabetic people by continuously monitoring their glucose levels. Heller aims his current work at alleviating suffering.

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., in Alameda, CA, where he led the development of *FreeStyle*, the first commercially available submicroliter blood glucose test strip, as well as the *FreeStyle Navigator* redox polymer-based continuous glucose sensor. He is Director of Advanced Development at Abbott Diabetes Care, the successor of TheraSense.

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