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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 33/487, 27/30, A61B 5/00

A2

(11) International Publication Number: **WO 00/10007**

(43) International Publication Date: 24 February 2000 (24.02.00)

(21) International Application Number: PCT/US99/18615

(22) International Filing Date: 16 August 1999 (16.08.99)

(30) Priority Data:

60/096,739	17 August 1998 (17.08.98)	US
09/301,252	28 April 1999 (28.04.99)	US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application

US 09/301,252 (CON)
Filed on 28 April 1999 (28.04.99)

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

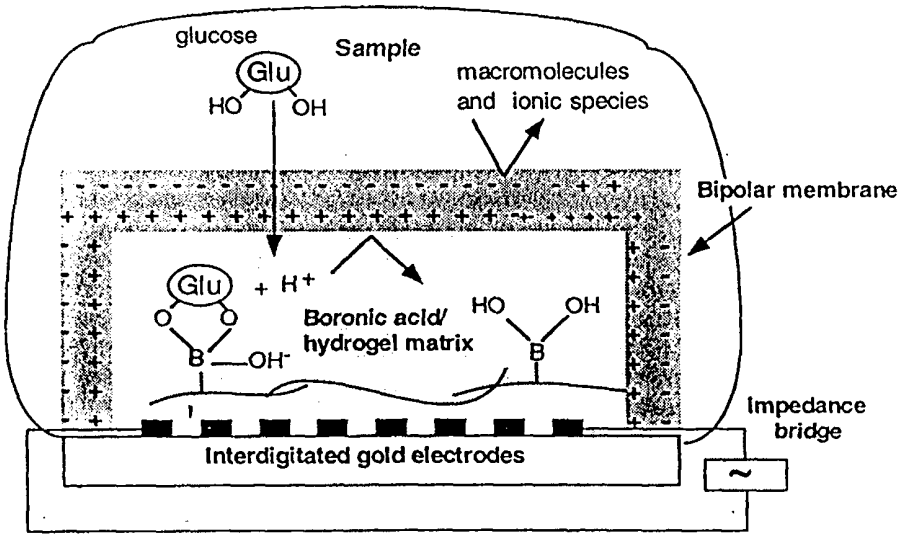
Published

Without international search report and to be republished upon receipt of that report.

(54) Title: DEVICES AND METHODS FOR ANALYSIS OF NON–IONIC SOLUTES

(57) Abstract

This invention relates to devices and methods for detecting and monitoring the concentration, level or amount of a target molecule in solution. More particularly, the invention is directed to sensors that evaluate changes in conductivity produced by the reaction of a target molecule with a sensor composition. The invention is particularly well suited for determining the concentration of carbohydrate compounds in solution, such as diol compounds, preferably biologically significant cis–diols such as various sugars including glucose.



The invention also encompasses sensors for determining the total or relative amount of target molecule in a sample, particularly a biological sample such as blood, urine, or other biological fluids. A laminated sensor of the invention comprises a sensor composition applied to a metal electrode, such as an interdigitated gold electrode on a non–conductive support. The sensor composition and electrode are and preferably encapsulated by a bipolar membrane. In a preferred embodiment, the sensor composition is a blend of a boronic acid polymer and a non–ionogenic hydrogel matrix.

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DEVICES AND METHODS FOR ANALYSIS OF NON-IONIC SOLUTES

The U. S. Government has certain rights in this invention pursuant to Grant No. BES-9416915 awarded by the National Science Foundation, and Grant No. N00014-92-J-1178 awarded by the U.S. Navy.

This application claims the priority of U.S. Patent Application No. 60/096,739 filed August 17, 1998.

All of the references disclosed herein are incorporated in their entireties.

FIELD OF THE INVENTION

This invention relates to devices and methods for detecting, analyzing, monitoring or measuring the concentration, level or amount of a target molecule in solution. Preferably, the target molecule is a non-ionic solute or microsolute in an aqueous solution. More particularly, the invention is directed to sensors for cis-diol compounds, preferably biologically significant cis-diols, such as various sugars including glucose. The invention also encompasses the use of these sensors to determine the total or relative concentration or amount of target molecule in a sample, particularly a biological sample. In a preferred embodiment, the target molecule is glucose and the biological sample is blood, urine, or another biological fluid.

According to the invention, the target molecule is exposed to a sensor composition, which comprises a complexing agent that causes a detectable change in ion concentration, *e.g.* hydrogen ion concentration [H+], acidity or pH, that is related or proportional to the concentration of target molecule. The target molecule can also be called a test compound or analyte. In its broadest aspect, the invention can be used to detect or quantify any target molecule, so long as contact or interaction with the sensor composition produces a related and detectable change in ion concentration. In certain embodiments the target molecule is non-ionic, such as a diol compound. The sensor composition comprises a boronic acid compound in some preferred embodiments. However, any sensor composition that having a complexing agent that captures or releases one

or more ions, or changes the concentration of mobile ions, in response to contact with a target molecule is suitable. For example, metal complexing agents can also be used, as described in U.S. Patent Application, Serial No. 08/875,047, incorporated by reference.

Interaction of a target molecule (such as glucose) with a complexing agent or sensor composition (such as a composition containing a boronic acid compound or a metal complex) liberates ions, such as hydrogen ions in the case of glucose and boronic acid. This changes the ion concentration in the local environment of the sensor, to a degree determined by the concentration of target molecule in solution. For example the H^+ concentration changes when the sensor composition comprises a boronic acid compound. This can be measured in many ways, including measuring a change in conductance, impedance, or pH.

In a preferred embodiment, the sensor composition comprises a complexing agent that is a polymeric aromatic boronic acid compound, such as poly(vinyl phenyl boronic acid). More preferably, the boronic acid polymer is combined or copolymerized with a matrix polymer, preferably a hydrophilic polymer, to form a sensor composition that is a water-absorptive and water-insoluble hydrogel matrix. A preferred hydrophilic polymer for the hydrogel matrix is poly(2-hydroxyethyl methacrylate). Typically, such compositions are formulated to contain about 25-75% boronic acid polymer, with the remainder comprising the matrix polymer and other ingredients such as stabilizers, preservatives, etc. Optionally, the matrix polymer or hydrogel may be imprinted with the target molecule, or an analog thereof, to improve selectivity.

The sensor composition can be used with an electrode for monitoring the presence and concentration of target molecule in a sample, *e.g.* a solution or biological fluid. For example, a sensor composition comprising a boronic acid polymer and a hydrogel can be applied, fixed or laminated to a solid support, such as a metal electrode, preferably a gold electrode, and most preferably an interdigitated gold electrode. In a preferred embodiment, a sensor is made by laminating a sensor composition (*e.g.* a combination of boronic acid polymer and hydrogel) to a metal electrode (*e.g.* an interdigitated gold electrode).

In a more preferred embodiment, the sensor composition, preferably comprising a hydrogel with a boronic acid component, is isolated from the target or test solution by a semipermeable barrier. The barrier is impermeable to ions, including ionic species in the solution and in the sensor composition. The barrier is permeable to the non-ionic target molecule and may be permeable to other non-ionic species. In this way, ions detected by the sensor, for example as

a change in pH, conductance, or impedance, are predominantly if not exclusively ions produced by interaction of the target molecule and complexing agent. This minimizes the influence of other potentially interfering ions on the sensor, improves the direct correspondence between ion concentration and target molecule concentration, and provides for more accurate sensor readings.

For example, the sensor composition can be sandwiched between an electrode and a membrane, preferably a bipolar ion exchange membrane. That is, the sensor and electrode are isolated from the external testing environment (*e.g.* a solution to be tested for the presence or concentration of target molecule) by an appropriately thin bipolar membrane. Ions in the target solution are excluded from the sensor composition by the membrane. Ions produced within the sensor composition by interaction with the target molecule, which does cross the membrane, are held within the sensor because they do not cross the membrane. Thus, the sensor accurately responds to the local ion concentration, which is a direct and reliable function of the concentration of target molecule in the test solution.

As the concentration of target molecule in the test solution changes, the target molecule is free to move in and out of the sensor composition, until an equilibrium is reached: the concentration of target molecule in the solution and in the sensor composition (on both sides of the membrane) is the same. In certain embodiments, a reading taken after a sufficient time for equilibrium to be reached gives an accurate indication of the concentration of target molecule in solution, as a function of ion concentration in the sensor composition. In other embodiments, readings may be taken without equilibration.

The interaction between a target molecule and a complexing agent in the sensor composition is preferably reversible, so that decreases as well as increases in the concentration of target molecule can be evaluated. For example, unreacted glucose and boronic acid compound in the sensor composition may reach an equilibrium with reacted glucose and boronic acid, plus hydrogen ions. If the glucose concentration is increased, more glucose binds to the boronic acid group, more hydrogen ions are released and measured, and a determination of the increased glucose concentration can be made. If the glucose concentration in the test solution is decreased, the boronic acid compound releases glucose in exchange for hydrogen ions. The free glucose diffuses out of the sensor composition, with a corresponding decrease in hydrogen ion concentration. Because of this dynamic equilibrium, sensor measurements used to determine glucose concentration are preferably taken when a stable equilibrium is reached. This can be

done, for example, by waiting a sufficient period for sensor readings to stabilize. Algorithms, internal standards, and other suitable means may also be used to calibrate the sensor and to improve accuracy and reliability. Note also that equilibrium can be reached more quickly, and analysis can be more accurate, when the size of the sensor, particularly the volume of the sensor composition, is small -- particularly in relation to the volume of test solution. Thus, small or microfabricated embodiments of the invention are preferred for certain applications.

The sensor may have any suitable shape. For example, a sensor composition can be applied to electrodes to form a wafer-like shape, which can then be covered, preferably on all sides, by a membrane. A wafer-like shape can also be formed into a roll. Alternatively, electrodes can be embedded in a sensor composition of any shape, and the sensor composition can be coated or covered with a membrane. A cylinder-like or tube-like shape can be used, particularly for small implantable devices, in which electrodes are coated with sensor composition in all directions (*i.e.* 360 degrees) around at least one common axis. A membrane can be applied to the sensor composition as an outer shell.

When the sensor composition is used with an electrode, the sensor is contacted with a sample (*e.g.* a solution or biological fluid) that is being tested for the presence or concentration of target molecule. The concentration of target (*e.g.* glucose) can be determined by measuring the conductance or impedance in an electric circuit formed within the sample and completed by the electrode, *e.g.* between adjacent surfaces of the fingers or digits of an interdigitated metal electrode.

Other techniques in addition to conductimetry may be used to detect or measure ion concentration, or the change in ion concentration, and thereby determine the concentration of target molecule. For example, a fluorophore can be used to produce a change in fluorescence as a way to determine a change in pH (fluorimetry). A color change reaction may also be used, for example by monitoring the color change of a chromophore in response to pH (colorimetry). Polarimetry is another alternative. Light scattering or nephelometry can be used when the released ion is reacted with another compound or reagent to produce an aggregate or colloidal particle. Specific ion potentiometry may also be used. A field effect transistor (FET), light addressable potentiometric sensor (LAPS), or combinations of these techniques may be used.

Sensors of the invention may be miniaturized, for improved accuracy and speed, and to minimize the amount of biological sample needed. Miniaturized sensors may also be used as

implantable devices, for example to continuously monitor blood glucose levels in a diabetic individual. Sensors of this type include minimally invasive devices, such as subcutaneous needle sensors and reverse iontophoresis sensors where glucose is extracted across the skin, for example using ultrasound.

BACKGROUND

Instrumentation for the rapid and accurate determination of the concentration of specific organic compounds present in gases or liquids is an important requirement in industrial process control, environmental monitoring, and clinical medical and diagnostic practice. Sensors which generate an electrical or optical signal in response to the presence of such a compound in the test medium are among the most widely used devices for this purpose. One common type of sensor is an ion-specific potentiometric sensor such as the glass pH electrode. *See* A. Lewenstam, *et al.*, *Electroanalysis*, **3**:727 (1991); D.M. Pranitis, *et al.*, *Crit. Rev.Anal. Chem*, **23**:163 (1992); K.L. Cheng, *ACS. Symp. S.*, 390: 286 (1989); M. Grattarola, *et al.*, *IEEE Trans. Electronic Devices*, **39**: 813 (1992). Another device uses the Clark oxygen electrode sensor. *See* C. Meyerhoff *et al.*, *The Endocrinologist*, **6**: 51-58 (1996); T.C. Kuchnicki and N.E.R. Campbell, *Analyt. Biochem.*, **149**: 111 (1985); R.R. Wise and A.W. Naylor, *Analyt. Biochem.*, **146**: 260 (1985). Spectrophotometric sensors are also known, including those which evaluate the reaction products of solutes with reagents present in the optical path, determine optical density, O.S. Wolfbeis, Ed., *Fiber Optical Chemical Sensors and Biosensors*, CRC, Boca Raton, Fl. 2, pp 267-300 (1991); E.V. Alonso, *et al.*, *TALANTA*, **43**: 1941 (1996), or measure the fluorescence of specific solutes. M.J.P. Leiner, *Analyt. Chim.*, **255**: 209 (1991); K. Sohanpal, *et al.*, *Sensors and Actuators B*, **11**: 547 (1993). Fiber-optic sensors are also known. *See e.g.*, W. Trettnak *et al*, *Biosensors* **4**: 15-26 (1988). A carbon dioxide optrode sensor is also known, B. H. Weigl *et al.*, *Analytica Acta*, **282**: 335-343 (1993), as is an optical sensor for measuring highly basic solutions. T. Werner and O.S. Wolfbeis, *Fresenius J. Anal. Chem.*, **346**: 564-68 (1993).

Other sensors, by optical or electrical measurement, determine the concentration of reaction products generated by the interaction of a target molecule with a highly-substrate-specific catalyst (e.g., enzyme) present in the sensor compartment. A. Seki, *et al.*, *Analyt. Chim.*, **373**: 9 (1998); H.Y. Aboulenein, and R.I. Stefan, *Crit. Rev.Anal. Chem*, **28**: 259 (1998). One example of this type of sensor is an enzyme-based glucose sensor (*e.g.*, using a glucose-oxidase assay) for

monitoring glucose concentration in biological fluids. G. Palleschi, *et al.*, *Appl. Biochem. Biotechnol.*, **31**: 21 (1991); S.M. Reddy, *et al.*, *Analyt. Chim.* **363**: 203 (1998). The known sensors have many disadvantages, including for example potential adverse reactions from the use of biological or enzymatic materials, drift in baseline measurements that lead to inaccurate measurements, high costs and complexity, etc.

There is a need for the accurate, rapid and inexpensive measurement of sugar or glucose levels in a variety of industries. For example, many industrial and food processing systems (*i.e.* fermentation) require that the level of one or more cis-diols or sugars be carefully monitored at various stages to insure the desired quality of final products. This need is especially acute in the medical context where accurate determinations of sugar levels (principally glucose) is critical for the proper diagnosis and treatment of various diseases, the most prominent of which is diabetes mellitus.

Affecting more than 14 million people in the United States and over 100 million people worldwide, diabetes is a disease of the metabolic system that is caused by a lack of the protein insulin, a key hormone for many animals including man. This insulin deficiency results in the inability of the body to self-regulate blood sugar levels, which can lead to a variety of other medical problems including blindness, heart disease, kidney failure, coma, and even death. Because the problems associated with diabetes may be prevented by maintaining glucose levels within a normal range, a cornerstone of any diabetes treatment protocol is the self-monitoring of blood glucose level. See, The Diabetes Control and Complications Trial Research Group, *New Engl. J. Med.* **329:** 977 (1993). For example, diabetes can be controlled by administering insulin when necessary -- but given the delicate balance of this biological feedback system, there is a need for improved techniques for knowing when insulin (or any other treatment) is actually needed. Indeed, the timing of insulin therapy is of major concern in controlling this chronic disease.

Currently available glucose sensors are generally based on the enzymatic conversion of glucose to its oxidized or reduced form. Pickup, J. *Trends in Biotechnology*,**11**: 285-291 (1993); D.A. Gough, *et al.*, *Diabetes*, **44**: 1005 (1995). Although these enzymatic sensors can be relatively simple to use and have the needed reproducibility, the costs associated with these enzymes and their inherent instability are some of the drawbacks to these systems. Poor stability

is of particular concern for implantable devices, as is the potential immune reaction to the foreign enzyme or its degradation products.

Various attempts have been made to find non-enzymatic alternatives for measuring glucose levels. Of these, boronic acid and their derivatives have shown promise because of their observed ability to form covalent complexes with various carbohydrates, particularly sugars including glucose. R. Consden and W.M. Stanier, *Nature*, **169**: 783-85 (1952); T.D. James, *et al.*, *Angew. Chem. Int. Ed. Engl.*, **35**: 1910-22 (1996); Y. Shiome, *et al.*, *J. Chem. Soc. Perkin Trans.*, 2111 (1993); J.C. Norrild, and H. Eggert, *J. Am. Chem. Soc.* **117:** 1479 (1995). When sugar binds to the boronic acid compound, protons are released, causing a change in pH. For example, phenyl boronic acid and its derivatives can form cyclic esters with the polyhydroxyl groups of sugars (saccharides) in aqueous solution S.A. Barker, *et al.*, *Carbohydrate Research*, **26**: 33 (1973). However, the binding mechanism is not well understood, nor is the behavior of boronic acid-sugar complexes in aqueous solution. The research effort has therefore focused on understanding the nature and behavior of this sugar-boronic acid complex. Some researchers have speculated about the potential uses of boronic acid compounds as sensors, if the reaction of sugars with these compounds could be suitably coupled to a means for generating a detectable signal.

A number of crude optical and electrochemical glucose and sugar sensors based on binding to boronic acids have been proposed. See, *e.g.* A. Kikuchi, *et al.*, *Anal. Chem.*, **68**: 823(1996); G. Wulff, *Angew. Chem. Int. Ed. Engl.*, **34**: 1812 (1995); K. Tsukagoshi, and S. Shinkai, *J. Org. Chem.*, **56**: 4089 (1991); T.D. James, *et al.*, *J. Chem. Soc. Chem. Commun.*, 281 (1996); S. Shinkai, *et al.*, *J. Chem. Soc. Chem. Commun.*, 1039 (1991). Most use additional functional groups attached to boronic acid for signal transduction. Their performance in complex biological samples such as blood or plasma has not been reported. Examples of these efforts include conjugating or attaching boronic acid groups to probes, such as chromophores and fluorophores, for detecting glucose using various forms of spectroscopy. T.D. James et al., *Angew. Chem. Int. Ed. Engl.* **35**: 1910-1922 (1996); S.B. Bambot, *et al., Sensors and Actuators B,* **22**: 181-188 (1994). These methods require synthesizing complex boronic acid derivatives and the use of expensive detection equipment. Practical, simple, and inexpensive approaches for measuring glucose have not been provided.

A cruder technique for detecting levels of glucose between 1000 mg/dL and 10,000 mg/dL is described in U.S. Patent Nos. 5,217,691 and 5,116,763. Briefly, these methods rely on the color change of various pH indicators to determine whether a predetermined level of glucose exists within a sample. Each pH indicator changes color at a particular threshold pH. When enough protons are released by the formation of boronic acid-sugar complex, the pH changes sufficiently to reach the threshold of an indicator, and a color change is observed. Typically, the pH decreases as the release of protons make the solution more acidic. A very rough estimate of the sugar level can be made from the color change. This method appears to detect glucose in concentrations of about 1000 mg/dL. Human blood glucose levels are typically between about 30 mg/dL and about 450 mg/dL. Thus, the prior art pH indicator method lacks sufficient sensitivity, readability, reproducibility and accuracy for use by diabetic patients to routinely monitor their blood glucose levels. In particular these methods are not well suited for continuous glucose monitoring.

Another method uses implanted glucose sensitive living cells (beta cells from the islets of Langerhans in the pancreas) to monitor blood glucose levels. Palti, *et al.,* U.S. Patent No. 5,101,814. The implanted living cells produce a detectable electrical or optical signal in response to changes in glucose concentration in surrounding tissue. The disadvantages of using living cells are immediately apparent. For example, the cells may produce an undesirable immune response. Additionally, the cells must be kept alive, in contact with, but isolated from the body tissue and fluids in which they are implanted, for example by placing them in a capsule that is permeable to nutrients but impermeable to antibodies.

Certain carbohydrates have been shown to complex with certain metals. S.J. Angyal, *Advances in Carbohydrate Chemistry and Biochemistry,* **47**: 1 (1989); K.B. Hicks, *et al.*, *Advances in Carbohydrate Chemistry and Biochemistry,*, 46 (1988). Another detection method of the invention can be based on this principle, and comprises the formation of a metal-complexing polymer that is observed to bind glucose and release protons in proportion to the glucose concentration over the clinically relevant range of 0 to 25 mM. In one approach of this kind, a glucose sensing polymer uses the binding of sugar to a copper (II) complex or chelate, *e.g.* Cu(II)-triazacyclononane, to generate a change in hydrogen ion concentration. Wilson, *Nature Biotech.* **15**: 322; Chen et al., *Nature Biotech.* **15**: 354-357 (1997); U.S. Patent Application Serial No. 08/875,047. This material produces the strongest signals at highly alkaline pH (more than

about 9 or 10). Although the metal-complex approach is promising the signal is substantially weaker at physiological pH (*e.g.* 6-8, especially 7-7.4).

The sensors in use today have limitations which restrict their utility for many important applications. These limitations include lack of durability or chemical or thermal stability; loss of response-accuracy or reproducibility due to contamination by extraneous solutes; high fabrication costs; and requirement for delicate and costly electronic or spectrophotometric readout-instrumentation. Another important limitation of many such sensors is their unsuitability for continuous monitoring of specific solute concentrations in fluid streams subject to frequent and/or rapid compositional changes (e.g., monitoring of toxicants in water supplies, of biological products in fermentation process streams, or *in vivo* monitoring of bioactive substances in flowing blood). Yet a further constraint on the utility of such sensors is their lack of adaptability to microminiaturization techniques of fabrication. Another disadvantage is that sensors containing biological materials such as enzymes may elicit an undesirable immune response or a toxic reaction. These materials may also be difficult to sterilize.

Conventional one-time or "spot" measurements require a blood sample, typically by pricking a finger, which for glucose monitoring must be done many time a day. Thus, there is also a need for improved methods and devices to monitor other fluids, such as saliva and urine, and for other ways to obtain samples. For example, transdermal extraction of samples may be used in concert with improved methods and devices herein, to provide more convenient, efficient and economical sensors. Continuous, semi-continuous, or regular monitoring for real-time management of glucose concentration is medically preferred, and can be combined with an insulin delivery system for improved therapy. Various methods have been investigated, including implantable devices. Known continuous monitoring devices are described for example in Pickup, J. *Trends in Biotechnology*,**11**: 285-291 (1993). These devices have disadvantages, however, in that they can be complex, costly, and cumbersome, and generally are enzyme-based. These enzyme-based sensors are not sufficiently stable. Thus, there is a continuing need for smaller, simpler, and safer devices and methods for continuous monitoring, including improved microfabricated and implantable devices.

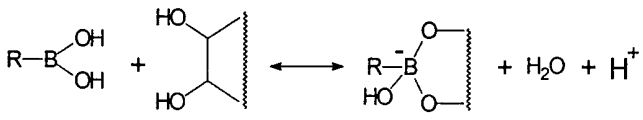
Consequently, there is a continuing need for new and improved sensors, and in particular for viable alternatives for measuring blood sugar levels, including continuous monitoring. The ideal methods would not use biological (*e.g.* poorly stable and/or immunogenic) materials, would

not require a complex chemical synthesis, would not depend on time-consuming or expensive manufacturing techniques, and would not rely on large, complex or expensive equipment. Such methods would be suitably specific for the desired target molecule such as glucose, would employ stable materials and reagents with a long shelf-life, would be stable during use, and would be accurate and simple to use. They would be insensitive to interfering compounds and environmental changes, and would be sensitive to changes in physiological amounts and concentrations of sugar.

SUMMARY OF THE INVENTION

The invention provides methods and devices for detecting and determining the amount or concentration of a target molecule in a sample, using a sensor composition comprising a complexing agent that releases ions when exposed to the target molecule. For analysis of cis-diol compounds, such as sugars, complexing agents, boronic acid compounds are preferred. Other suitable sensor compositions comprise metal complexing agents. Chen et al., *supra.* and U.S. Patent Application, Serial No. 08/875,047. In a preferred embodiment, a change in pH is observed when a boronic acid compound is exposed to a diol compound such as a sugar (*e.g.* glucose). The boronic acid and sugar compounds each terminate in two hydroxyl (OH) groups. These groups interact to form a sugar-boronic acid complex, with the release of a water molecule (H_2O) and a hydrogen ion (also called a proton, or H^+). The H^+ ion can combine with water (H_2O) to form a hydronium ion (H_3O^+). The resulting ions can be detected by any method, including measuring changes in pH, conductance, or impedance due to these ions. This in turn can be used to detect and measure the concentration of sugar in a sample, by exposing the sample to a boronic acid compound and observing the change in ion concentration, *e.g.* by conductance.

The reaction between a diol and a boronic acid compound can be represented as follows:



In this reaction scheme, B indicates a boron atom, and R indicates any substituent bonded to the boron atom.

The binding of diols to boronic acids generates hydrogen ions in a specific pH range that depends on the pKa of the specific boronic acid. Greene, *et al.*, U.S. Patent No. 5,217,691 (1993). The pKa of phenyl boronic acid, for example, is 8.86. Binding to glucose leads to a measurable change in pH in the range of about 6.5 to 10. Thus, glucose binds to phenyl boronic acid and liberates hydrogen ions in relationship to the glucose concentration at physiological pH.

The invention provides a molecular sensor, and in one embodiment a glucose sensor, which exploits these principles. Glucose concentration can be correlated with the change in AC impedance of a thin layer of a glucose-permeable hydrogel containing a boronic acid compound,

such as poly(vinyl phenyl boronic acid), deposited on the surface of a pair or pairs of planar electrodes when exposed to a glucose-containing aqueous solution. An interdigitated electrode can be used, wherein the current between adjacent fingers or pairs of digits on the electrode are monitored.

For the preferred glucose sensor applications, the sensor should contain a high enough mass-concentration of complexing agent (*e.g.* boronic acid groups) to assure a significant response from the release of ions (*e.g.* hydrogen ions) upon glucose complexation. The sensor composition should have sufficient mechanical strength and film-forming properties to allow a uniform and durable deposition onto an electrode substrate. It should be sufficiently permeable to water to allow free diffusion of water-soluble solutes, with a minimal impact on the mobility of ions in an electric field. The sensor composition should have a sufficiently low intrinsic water solubility and a high chemical stability in an aqueous environment, to avoid dissolution or decomposition in the course of the normal lifetime of the sensor.

By themselves, polymeric boronic acid compounds such as poly(vinyl phenyl boronic acid) may not provide all of these desired properties. For example, homopolymeric poly(vinyl phenyl boronic acid) has adequate chemical stability and boronic acid content (measured in mmols/ml dry polymer), but is too lipophilic (oily) to optimally absorb water and species such as glucose, which have a high water solubility and mobility. Also, this polymer has a relatively low molecular weight and high rigidity, and low mechanical strength. This makes it difficult to cast the polymer from solution as a thin film with good substrate-adhesion and resistance to cracking, chipping or splintering in an aqueous environment.

These physical and chemical properties can be improved by blending or copolymerizing the complexing agent (e.g., a boronic acid polymer with a matrix or hydrogel-forming-polymer, to form a hydrogel). For example, a boronic acid polymer can be mixed or reacted with a non-ionic, hydrophilic, linear polymer that has low water-solubility and a relatively high ability to retain water at ambient temperature. Suitable hydrogel polymers are known. N.F. Sheppard, *et al.*, *Sensors and Actuators B*, **10**: 73-77 (1993). One such polymer is linear poly(2-hydroxyethyl methacrylate) or poly(HEMA). This polymer is an excellent film-former, is water-insoluble, and can reversibly absorb approximately 50 % by volume of water at ambient temperature. It is soluble in ethanol and many other polar organic solvents, from which uniform, defect-free films and coatings can readily be cast. Poly(vinyl phenyl boronic acid), which is most readily soluble

in relatively low-polarity solvents such as toluene, methylethyl ketone, and methylene chloride, is also soluble in certain aprotic amphiphilic solvents such as N-methyl pyrrolidone (NMP). NMP is also a suitable solvent for poly(HEMA). Thus, homogenous solutions of these two polymers can be produced in this solvent, for example containing the boronic acid polymer and hydrogel polymer in a 1:3 weight ratio. Continuous transparent films can be made by casting this solution on a solid substrate (*e.g.* a metal electrode) followed by solvent evaporation. These films comprise a hydrogel matrix to contain the polymeric boronic acid compound, have excellent substrate-adhesion, and are capable of absorbing water from aqueous solution to a level of about 50 % by volume.

In one embodiment, the thickness of the hydrated matrix (boronic acid polymer and hydrogel polymer) is estimated to be about 20 micrometers, with a volume of ca. 800 nanoliters. This material forms a sensor composition or sensing layer of the glucose sensor, and provides the "control volume" of the sensor. For most measurements, the ratio of the test solution volume to the sensor "control volume" is so large that the change in solution composition resulting from solute migration into the matrix layer can be considered to be negligible.

Sensor Composition

According to the invention, a sensor composition comprising a complexing agent, such as a boronic acid compound, can advantageously be formed as a polymer or copolymer. The polymer or copolymer can be imprinted with a template, typically the target molecule or an analog thereof. The template is removed before the polymer is used as a sensor. This leaves spaces in the polymer that tend to conform to the shape of the target molecule, which improves the ability of the polymer to bind the target molecule. The complexing agent can also advantageously be combined with a matrix polymer, such as a hydrophilic or hydrogel-forming polymer. The matrix provides a support structure for the complexing agent and in particular improves its use in a diol or sugar sensor when a boronic acid compound is used as a complexing agent. Preferably, the complexing agent is immobilized and confined by the matrix and does not migrate or leach into the surrounding environment.

In a preferred embodiment of the invention, the boronic acid compound is provided as a polymer that is combined with a hydrogel matrix material, such as a hydrophilic polymer. A preferred boronic acid polymer is poly(vinyl phenyl boronic acid) and a preferred hydrophilic polymer is poly(2-hydroxyethyl methacrylate). The polymeric boronic acid compound and

hydrophilic polymer form a preferred sensor composition or complexing agent, which may also include other ingredients such as stabilizers and preservatives.

Electrode

For use as a diol or sugar sensor, the sensor composition is applied in a thin layer to a metal electrode, preferably an interdigitated gold electrode. The sensor composition may be placed between the electrode and a thin membrane, to form a laminated or layered composite electrode or sensor. Optionally, the edges or periphery of the laminate can be sealed by the membrane, to prevent leakage of solutes between the hydrogel matrix layer and the test solution. In a preferred embodiment, the target molecule is glucose, and the boronic acid polymer is provided in a known volume, amount or concentration.

Electrical conductivity is among the simplest of physicochemical measurements that can be performed upon an aqueous mixture of solutes. All that is required is a pair of electronically-conductive electrodes (also collectively called "an electrode") that is maintained in contact with a fixed volume of solution. A low-voltage source is applied across the electrodes, and a microammeter can be used to measure the current passing through the solution in the presence of the applied voltage. The electrical conductivity of the solution is determined by the concentration, mobility, and valency (charge) of ionic species present, corrected for any ion-polarization or electrolytic processes occurring at interfaces of the electrode and the solution. If a low-voltage AC potential in the audiofrequency range (e.g., 1 kHz) is employed, conductance interferences attributable to polarization and electrolysis are virtually eliminated, and under these circumstances the measured AC impedance of the solution is essentially its pure resistance, which is the reciprocal of the conductance of the bulk solution. Brett and Brett, *Electrochemistry Principles, Methods, and Applications*, Oxford University Press, p. 224 (1993); N.F. Sheppard, *et al.*, *Sensors and Actuators B*, **10**: 73-77 (1993).

Solution conductance depends on the sum of the contributions of all mobile ions present. That is, simple conductance is a measure of the total concentration of ions in equilibrium in the solution. Therefore, a suitably accurate detection or measuring method based on conductance must be sensitive predominantly or only to those ions of interest, for example by avoiding or screening out other ions. A material which has no electrical charge (it is not ionized) will generally not affect conductance, and can not readily be detected or measured in this way.

According to the invention, a non-ionic target molecule or substrate material can be detected or measured by conductance, if it is reacted with a known or excess concentration or amount of complexing agent, to liberate an ion or form a specific ionized product. In this scenario, the ionized product is produced in proportion to the concentration (amount/volume) of target molecule or substrate material. Measured changes in conductance produced by that product give an indication of the concentration of target or substrate. Stated another way, the increment in conductance resulting from the reaction will be directly related to the concentration of the target or substrate initially present, and to the fraction of the target or substrate that has formed the product. If the mobility and charge of the ions generated in this reaction are known, then the concentration of the target or substrate can be determined. Alternatively, the change in measured conductance can be compared to a calibration curve, e,g. The change in conductance for known glucose concentrations. The accuracy of this determination will depend on the magnitude of the solution conductance attributable to the ionic species present initially (the "background conductance"), relative to the increment in conductance resulting from the reaction. The lower the background conductance, the more accurate the determination. Any ion can be released, according to the invention, provided it is released in proportion to or as a function of the target molecule or analyte concentration.

Conductivity measurements can thus be applied as a generic strategy for determining the concentration of any non-ionic solute present in a multicomponent aqueous solution, provided that the solute can be reacted with a complexing agent to rapidly yield a mobile ionic product. One example, according to the invention, is the reaction of a carbohydrate containing compound, such as a sugar (preferably glucose) with a boronic acid compound or reagent. S.A. Barker, *et al.*, *Carbohydrate Research*, **26**: 33 (1973); T.D. James, *et al.*, *Angew. Chem. Int. Ed. Engl.*, **35**: 1910 (1996). This reaction releases mobile hydrogen ions, which measurably influence conductance. Another example is the formation of a complex between a sugar and a metal complexing agent (*e.g.* a metal bound to a ligand), which results in the production of an ion.

In preferred embodiments where conductivity is used to determine the concentration of a non-ionic target molecule, the complexing agent may be ionic, but other components of the sensor composition, such as a matrix or hydrogel component, are preferably non-ionic, in order to minimize the possible presence or effect of any stray ions.

The functionality of these sensors and methods, including their reliability and durability, and their practical utility for some applications, is improved or optimized by: (1) immobilizing the conplexing agent on the surface of the electrodes; and (2) interposing, between the complexing agent and the test solution, a membrane which is freely permeable to the non-ionic target-solute, but is essentially impermeable to all ionic species present in the test solution.

Applying these principles, the sensor composition and electrode of the invention are used to measure impedance in a sample, *e.g.* a sample to be tested for sugar content. The electrode is connected to an AC electrometer or impedance bridge, to permit measurement of the conductance or impedance of the sensor composition, preferably immobilized in a hydrogel matrix. This provides an indication of the concentration of protons in the sensor composition, which is a function of and provides an indication of the concentration of diol or sugar in the sample. When used, the membrane serves to shield the sensor composition and electrode from interference, which may be caused by the presence of other materials in the sample, or by loss or exchange of ions into the surrounding solution.

Selectively Permeable Membrane

When glucose in a test solution is contacted with a sensor composition, for example a hydrogel matrix comprising a boronic acid polymer as a complexing agent and a hydrophilic polymer, the liberated hydrogen ions are free to exchange with other cations present in the test solution. Other ionic species (including buffering electrolytes) are also free to enter the matrix. Consequently, the ionic conductivity of the hydrogel layer will be strongly dependent on the ionic composition of the test solution, its buffer capacity, and the relative volumes of the test solution and the boronic-acid-containing matrix, as well as on the glucose concentration.

When the test solution volume is not much greater than the "control volume" of the sensor, a knowledge of the ionic strength of the test solution, pH, buffer capacity and volume would make it possible to correct for indifferent ion contributions to conductivity. That is, once can correct the measurements to account for the changes in pH attributable to hydrogen ion liberation, and extract a reasonably accurate estimate of the glucose concentration from the measured sensor impedance or conductance, *e.g.* when a reference measurement of the baseline pH (without complexing agent) is made.

When the volume of the test solution is large compared with the volume of the matrix, it can be difficult or impossible as a practical matter to correct for these solution effects. In some

circumstances, the conductance of the sensor composition could nearly equal the conductance of the bulk solution it is contacting, irrespective of its glucose content. One solution to this problem is to interpose a semi-permeable membrane, between the boronic-acid-containing matrix and the test solution, that is impermeable to ions (including hydrogen and hydroxyl ions), yet is freely permeable to non-ionic species such as glucose. Membrane structures of this kind are commercially available, and include bipolar ion exchange membranes originally developed for use in bipolar electrodialysis (ED) or "water-splitting" applications for the production of acids and bases from neutral salts. These membranes are typically a laminate of a high-exchange-capacity cation exchange membrane with a high-exchange capacity anion exchange membrane. Through Donnan co-ion exclusion, the membrane prevents penetration and diffusion of ons and cations, except when subjected to high transmembrane direct current (DC) potential differences that are sufficient to split water molecules into hydrogen and hydroxyl ions at the interlayer boundary. These membranes are highly hydrated, and are freely permeable to low-molecular weight, non-ionic solutes of high water solubility such as glucose. The membrane may also be provided in the form of hollow fibers.

This type of membrane can be used to protect the sensor composition from hydrogen ion loss and/ or extraneous ion invasion from the test solution. This permits the use of conductance as a direct and particularly accurate measure of glucose concentration, irrespective of the composition or volume of the test solution. Nevertheless, a membrane, though preferred, is not necessarily essential, especially in circumstances where a small sample volume is used. For example, at least some benefits of using a membrane may be achieved in a microfabricated system that uses the alternative of sampling a small and defined volume of test solution. Thus, embodiments without a membrane typically will employ alternative ways to account for interference from other ions or environmental factors, such as the use of a reference electrode to measure background ion concentrations.

In preferred embodiments, particularly reusable or implantable (*e.g.* continuous) sensors, it is important for the sensor to be reversible. That is, the reaction of target molecule and complexing agent should be reversible. Stated another way, the reaction is preferably (though not necessarily) a dynamic equilibrium that is shifted toward or away from the production of an ion-forming complex, depending primarily on the concentration of target molecule under particular test conditions. In this way, decreases in the concentration of target molecule will cause

dissociation of the ion-forming complex, and "recapture" of released ions, with a resulting detectable or measurable change in ion concentration showing the change (increase or decrease) in target molecule concentration over time, or under different conditions. As one example, a sensor placed in a test solution containing the target molecule should respond to the target molecule to produce a measurement as described, and in addition, should respond accordingly when placed in a solution that does not contain target molecule. That is, the sensor composition should return to a baseline ion level that indicates the absence of target molecule, or indicates a standard or reference concentration of target molecule from which a change in target molecule concentration can be reliably and repeatedly measured. This feature of the invention permits equilibration and/or standardization of the sensor, and provides a convenient, efficient, and inexpensive continuous or reusable device.

Definitions

The terms used in this specification generally have their ordinary meanings, in the context of the invention, and in the specific context where each term is used. Certain terms are discussed below, or elsewhere in the specification, to provide additional guidance to the practitioner in describing the devices and methods of the invention and how to make and use them. For convenience, certain terms are highlighted, for example using italics and/or quotation marks. The use of highlighting has no influence on the scope and meaning of a term; the scope and meaning of a term is the same, in the same context, whether or not it is highlighted. It will be appreciated that the same thing can be said in more than one way. Consequently, alternative language and synonyms may be used for any one or more of the terms discussed herein, nor is any special significance to be placed upon whether or not a term is elaborated or discussed herein. Synonyms for certain terms are provided. A recital of one or more synonyms does not exclude the use of other synonyms. The use of examples anywhere in this specification, including examples of any terms discussed herein, is illustrative only, and in no way limits the scope and meaning of the invention or of any exemplified term. Likewise, the invention is not limited to the preferred embodiments.

The terms "*target molecule*", "*target*" or "*analyte*" mean any molecule, compound, substance or composition of matter that is identified by or chosen to be detected, measured, analyzed or in any way evaluated by using a method or device of the invention. Target molecules include those which produce a detectable change in ion concentration, acidity or pH when

exposed to a *complexing agent* or *sensor composition* of the invention. Target molecules include, without limitation, "*diol compounds*"and "*cis-diol compounds*", particularly when the complexing agent is a boronic acid compound. A diol is any compound or portion of a compound that has two hydroxyl (-OH) groups. A cis-diol is a diol compound in which the local two hydroxyl groups are in a cis relationship to each other, meaning that they both lie on the same side of a reference plane in the molecule. Suitable target molecules of the invention also include polyols, carbohydrates, and especially sugars. Exemplary target molecules include glucose, mannose and other monosaccharides, sialic acid, aminosugars such as glucosamine, disaccharides, trisaccharides, oligosaccharides, sugar-amino acid complexes, sugar-peptide complexes and glycoproteins (*i.e.* glycosylated proteins). When a metal complexing agent is used, the target molecule also may include for example glycerol; dopamine; catechols; ascorbic acid; 1,4, anhydroerythritol; ethyleneglycol; and 3-hydroxy-L-tyrosine. The target molecule can be a component of a liquid or gas, e.g. a volatile or non-volatile solute dissolved in a liquid.

An "*acid*" is a compound or molecule having a tendency to lose one or more protons, or gain one or more electrons, typically by sharing the protons or electrons with another molecule called a "*base*". The terms "*acidic*" and "*acidity*" refer to the tendency of a compound or molecule to behave as or have characteristics of an acid, including having the property of releasing one or more protons in aqueous solution. For example, an acidic solution is a solution that has a pH of less than 7.0.

The term "*pH*" has its conventional meaning in the art, and in broad terms indicates a relative measure of the concentration of hydrogen ions [H^+] in a solution, *i.e.* the negative common logartihm of the hydrogen ion concentration, expressed in gmol/liter. The pH of pure water at room temperature is 7.0, or "neutral" pH. Under these conditions the concentrations of hydrogen and hydroxide ions are equal. A solution having a pH of less than 7.0 contains a higher concentration of hydrogen ion and therefore is acidic. A solution having a pH of more than 7.0 contains a lower concentration of hydrogen ion and is therefore basic. Physiological pH is a range of pH above and below 7.0 that is generally suitable for living organisms, and ranges from about 6.8 to about 7.8, and preferably is about 7.4.

A "*complexing agent*" is any molecule, compound, substance or composition of matter that interacts with a target molecule to produce detectable ions, or a measurable change in ion concentration.

For example, a complexing agent comprises a compound having one or more groups that react with a target molecule to produce one or more ions. A preferred complexing agent comprises a boronic acid compound, preferably a polymer containing or comprising a boronic acid compound. Metal complexing agents may also be used. The selectivity or specificity of the complexing agent for the target molecule may be relative, need not be absolute, and depends for example on the target molecule and on the test sample and sensing conditions. A complexing agent may interact with more than one target molecule, and may produce ions in response to more than one molecule. For example, a sensor to be used for monitoring glucose in blood may have a complexing agent that also recognizes other monosaccharides. This is because the concentration of glucose in the blood is sufficiently greater than the concentration of other monosaccharides. Even in diabetic patients, where glucose levels may be lower than normal, the production of ions by interaction of other monosaccharides with the complexing agent is small enough to be disregarded or readily accounted for. In addition, more than one complexing agent may be used, each with a different specificity or selectivity for one more more target molecules or analytes. Using known methods and algorithms, the responses produced by a two or more complexing agents can be coordinated or deconvoluted to provide a more accurate measure of the concentration of particular analytes.

A "sensor composition" is any molecule, compound, substance or composition of matter that comprises, includes, or contains a complexing agent. The sensor composition may include or be accompanied by other ingredients, such as other polymers, matrix materials, stabilizers, preservatives, buffers, etc. The sensor composition may be in any form, such as solid, liquid, or gel, and in preferred embodiments is a hydrogel. The agent or composition may be formulated with other materials, such as polymers, to provide structural support or desired physical or mechanical properties, including for example hydrogel-forming polymers. In certain embodiments, two or more boronic acid compounds or components may be used together to provide a sensor composition, and in a preferred embodiment, the sensor composition comprises a boronic acid polymer or copolymer, especially an aromatic boronic acid polymer as a complexing agent. Also in a preferred embodiment, the sensor composition is provided as a hydrogel, for example comprising a complexing agent that is blended or reacted with a matrix or hydrogel-forming polymer.

The sensor composition may be applied or affixed to a solid support, such as a metal electrode, silicon, glass, quartz, ceramics, organic or inorganic polymers, zeolites, and other inorganic materials. The support can have any structure to which a complexing agent (*e.g.* a boronic acid compound) can be affixed or immobilized, such as beads, particles, membranes, plates, threads, fibers, and solid state devices such as FET and LAPS devices.

A "*boronic acid compound*", derivative or component means boronic acid itself, $HB(OH)_2$; and/or any compound or material having a boronic acid group, $-B(OH)_2$. That is, a boronic acid compound or component is any molecule having at least one boron atom (B) bound to a carbon atom of an organic moeity (R) and to two hydroxyl groups (OH), which can be represented as $R\text{-}B(OH)_2$. Symbolically, the letter "B" may be used to represent a boron atom in chemical formulae, as shown above. Elsewhere, for example in kinetic equations, the letter "B" may be used to represent a boronic acid compound as defined herein. The boronic acid compound may have a linear (straight-chain) structure, an aromatic (ring-like) structure, or both. The boronic acid compound or component may also be provided in the form of a polymer or copolymer. Aromatic boronic acid polymers are preferred. Non-limiting examples of suitable boronic acid polymers include poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid). A preferred polymer is poly(vinyl phenyl boronic acid).

A "*metal complexing agent*" is any molecule, compound, material or substance that comprises one or more coordinating metal ions joined to one or more other components or ligands (preferably polymeric), to form an association or complex which interacts or binds with a target molecule to produce one or more ions. The ions can be produced by any mechanism, provided there is a relationship, correspondence, or stoichiometry between the concentration of target molecule and the concentration of produced ions.

For example, one or more of the ligands held by the metal ion may be released in exchange for binding the target molecule. Alternatively, the target molecule may release one or more ions (*e.g.* a hydrogen or hydroxide ion) in exchange for binding with the metal complexing agent. Metal chelating agents may be used that bind a metal relatively strongly (*e.g.* copper), while leaving one or more binding sites available (*e.g.* one or more copper coordination sites) for exchange with a weaker ligand (an "*exchange ligand*", *e.g.* water). Such metal chelators can be

used as metal complexing agents when, under certain conditions such as pH, a target molecule is able to interact with the metal ion to produce a detectable or measurable change in ion concentration. For example the interaction may release hydrogen ions. The target molecule may displace another ligand from the complex, such as water. Preferred metal complexing agents provide at least two metal coordination sites which are free or can become available to bind a ligand that exchanges with a target molecule. The preferred metal is copper, however any metal may be used, provided that (a) the target molecule rapidly and reversibly interacts or binds with the complex formed by the metal ion and exchange ligand in the presence of a test sample; and (b) the interaction produces a hydrogen ion, hydroxide ion, or other detectable ion or substance.

One exemplary metal complexing agent is a triazacyclononane-copper II complex (copper(II)-triazacyclononane, Cu(TACN) or TACN-Cu^{2+}). At a pH of more than 9, this complexing agent strongly interacts with glucose with the net release of a hydrogen ion. This and other metal complexing agents can be incorporated into a polymer for use in a molecular sensor of the invention.

A "*ligand*" is any compound, molecule or substance that interacts, binds, or associates with another compound molecule or substance. For example, a target molecule can be referred to as a ligand for a complexing agent herein, and *vice versa*. In the context of a metal complexing agent, a ligand is also a compound, molecule or substance that binds a metal ion.

A "*polymer*" is any substance comprised of small molecules that are linked together in repeating units called "mers", and here includes any compounds having two or more "mers". The small molecules are called "*monomers*", which are chemically linked together by the process called

"polymerization". A "*copolymer*" is a polymer formed by the copolymerization of two or more chemically different monomers. As used herein, the broad term "polymers" generally includes copolymers.

Polymers of the invention may be "*imprinted.*" This means forming the polymer in the presence of one or more compounds or molecules which can provide a template for the polymer. A template compound influences the resulting structure or characteristics of the polymer, but does not itself make up a significant part of the polymer. According to the invention, a target molecule or analog thereof may be used as the template compound to imprint a polymeric complexing agent or a matrix polymer, or both.

A "*matrix*" polymer or material means any molecule, compound, substance or composition of matter that is water-soluble and water-absorbant, and which provides support or structure to the complexing agent or sensor composition (*e.g.* a boronic acid compound or polymer), or is a carrier for the complexing agent in a sensor composition. A matrix material together with a complexing agent, such as sensor composition formulated as a hydrogel, can be called a "*sensor matrix*". Inert supports materials are preferred, and hydrogel compositions are particularly suitable. A "*hydrogel*" is any gel in which water is the liquid component. A gel is a dispersion of a solid material in a liquid that has the appearance or semblance of a solid. Any suitable hydrogel may be used in the invention. In embodiments where the target molecule is dissolved in water (*e.g.* an aqueous solution), the hydrogel should be water-insoluble, and stable against decomposition in aqueous environments. In a preferred glucose sensor embodiment with a boronic acid complexing agent, such hydrogels include those made from hydrophilic polymers which do not have a terminal hydroxyl groups that may compete with glucose for reaction with boronic acid groups. Examples include poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether). A preferred matrix polymer is poly(2-hydroxyethyl methacrylate).

A "*stabilizer*" is any compound, molecule or additive that improves the ability of a composition or material to resist stress, for example heat, or which prolongs the useful life (*e.g.* the shelf-life) of a material or composition of matter. Suitable stabilizers include nontoxic antioxidants, and UV absorbers, for example nonionic compounds such as BHA or BHT, hexylresorcinol, and quininoid UV absorbers.

A "*preservative*" is any compound, molecule or additive which acts as a sterilizing agent, antiseptic, fungicide or bacteriocide, or which improves the ability of a material or composition of matter to remain sterile, or free of microorganisms. For example, phenolic compounds such as phenol and m-creosol may be used as preservatives.

An "*electrode*" is any conductor, typically metal, that is used to establish an electrical contact with a solution or with a less conductive material, and includes without limitation metals and metals that are covered with a metal salt, carbon, glass, or a membrane. Electrodes are often provided in pairs, with an electric current or current potential between them, where one electrode is an "*anode*" and the other is a "*cathode*". The anode is the positive electrode of the pair, and

is the electrode toward which negatively charged ions (*e.g.* electrons) migrate. The cathode is the negative electrode of the pair, and is the electrode toward which positively charged ions (*e.g.* protons) migrate. The term "*electrode*" may also be used to indicate at least one electrode pair, *i.e* an anode and a cathode. An "*interdigitated*" electrode is any electrode that has more than one pair of opposing electrodes or digits (fingers) that form anode and cathode surfaces, typically in a miniature pattern or array.

An *"ion"* is any compound or molecule, or portion thereof, also called an *"ionic species"*, that carries a positive or negative electrical charge under given conditions. A "*non-ionic*" molecule, compound, species or substance does not have an electrical charge under given conditions. The terms "*ionic"* and *"non-ionic*" can also be used, in context, to mean "*ionogenic*" and "*non-ionogenic*", respectively. That is, an ionic or ionogenic compound produces ions under certain conditions. A non-ionic or non-ionogenic compound does not produce ions under certain conditions.

A "*membrane*" is any thin sheet or layer separating two compartments or compositions. A "semipermable" membrane is a membrane that allows some compounds, molecules, ions or species to preferentially cross the membrane, whereas others do not. An "*ion exchange*" membrane is a semipermeable membrane that is relatively permeable to ions or species of like charge (*e.g.* positive ions), and is relatively impermeable to ions or species of unlike charge (*e.g.* negative ions). A "*bipolar membrane*" is a bonded laminate of an anion-exchange and cation-exchange membrane. Since the anion exchange layer is freely permeable only to anions, while the cation exchange layer is freely permeable only to cations, the laminate is virtually impermeable to ions of either sign-of-charge.

The term *"conductance"* has its conventional meaning in the art, and is a measure of the ability of a solution, e.g., an electrolytic solution, to carry an electric current via the movement of ions. It is also the reciprocal of electrical resistance.

The term "*impedance*" has its conventional meaning in the art, and can broadly be summarized as a measure of the opposition to current flow in an electrical circuit for a given current potential.

A "*sample*" means any material to be tested for the presence of a target molecule. Typically, the sample is a liquid, preferably a solution, or "*test solution.*" Most preferably the sample or test solution is an aqueous solution, meaning that the sample contains one or more

ingredients mixed or dissolved in water. In preferred embodiments the ingredients include a plurality of ionic and non-ionic compounds. For example, a typical sample or test solution according to the invention is a biological fluid, such as interstitial fluid, blood, saliva or urine.

The term "*alkyl*" refers to a linear or straight-chain (non-aromatic) hydrocarbon compound. Illustrative examples include but are not limited to alkanes, alkenes, and alkynes.

The term "*aryl*" refers to aromatic (ring-containing) compounds and is intended to include both aromatic hydrocarbons and aromatic heterocycles (*i.e.* furan, pyrrole and thiophene).

A substituted alkyl or aryl is one in which any atom or group (a *"substituent"*) replaces or can be considered to replace a hydrogen atom in a corresponding position of the unsubstituted aryl or alkyl compound. Illustrative examples of substituents and substitutions include but are not limited to nitro, halogen, C_1-C_5 alkyl, C_1-C_5 alkoxy, phenyl, hydroxyl, thiol, thioether, ketone, aldehyde, epoxy, ester, ether, amine, imine, amide, nitro, carboxylic acid, disulfide, carbonate, isocyanate, carbodiimide, carboalkoxy, and carbamate.

The term "*complex*" means any association of two or more chemical compounds or components that occurs when they are exposed to or brought in contact with each other. The term encompasses any and all interactions or chemical bond between the components, so long as there is an indication of some association, aggregation, or joining of two or more compounds or components that is detectable or distinguishable from the individual components themselves. A complex may form transiently, reversibly or irreversibly, and may be exist in equilibrium with other complexes or with compounds or components that are not part of a complex.

The term "*concentration*" means a measure of the mass or mole density of a specified substance or component of a solution or mixture, for example its mass (*e.g.* in grams or moles) per unit volume of the system in which it is contained (*e.g.* per cubic meter or per liter). As used herein, the concentration of a substance in a solution or mixture can be measured using any suitable method. Concentration can be expressed in any suitable form using any suitable units, including without limitation concentrations expressed as molar concentrations (M), normal concentrations (N), as a percentage of other components (*i.e.* % w/v or % v/v), or as a measure that is relative to a given standard or baseline concentration. The concentration of a species, ion, solute, compound, molecule or substance can be expressed symbolically by brackets. For example, where H^+ indicates hydrogen ions, $[H^+]$ indicates the concentration of hydrogen ions.

The amount of a substance can be derived or calculated from its concentration, by known means, for example when the volume of a sample is known.

A "*solute*" is a molecule, compound, ion or any substance that is present or is a component or ingredient of a liquid, called a "*solvent*", and typically is dissolved in the liquid. Preferably, the liquid is water, and the solute is therefore in an aqueous solution. A "*microsolute*" is a solute having a relatively low molecular weight, for example a small molecule having a molecular weight of less than 1,000, preferably less than 500.

The term "*equilibrium constant*" (usually symbolized by "K") is a mathematical quantity that relates the concentrations of reactants and products of a specified reversible chemical reaction taking place in a given reaction medium after the reaction has proceeded to completion at specified

environmental conditions (e.g., pressure, temperature, solvent, etc.). For example, for the reaction $A + B \rightleftharpoons C + D$, the equilibrium constant is defined by the relationship $K = [C][D] / [A][B]$, where the quantities in brackets are the molar concentrations at equilibrium of products and reactants, expressed in consistent units. At equilibrium, there is no further change in mixture composition with time, meaning that the concentrations of products and reactants do not change with time. The larger the value of (K), the more favorable is the forward reaction, and vice versa. Under these conditions, the mixture considered to be at "steady state", meaning that the rates of the forward and reverse reactions are equal and opposite. In kinetic terms, the equilibrium constant is the ratio of the rate constant for product formation from the reactants, divided by the rate constant for reactant formation from the products.

The terms "*association constant*" and "*dissociation constant*" apply to the equilibrium constants of chemical reaction in which two or more reactant molecules react to form a lesser number of product molecules, or vice versa. For example, for the reaction $AB \rightleftharpoons A + B$, the equilibrium (or dissociation) constant is defined by the relationship $K = [A][B] / [AB]$. For any specific reaction, the association constant and dissociation constant are reciprocals of one another. If an association or dissociation reaction yields products (or consumes reactants) which carry electrical charge, then the dissociation or association constants are frequently called "*ionization constants*".

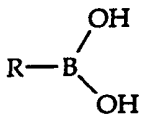
The term "pK" is a symbol for the reciprocal of a dissociation constant, expressed on a logarithmic scale, *i.e.* $pK = \log 1/K$. The type of constant may be indicated by the subscript, *e.g.*

pK_a is the dissociation constant of an acid, $pK_{A(BH)}$ is the acid dissociation constant of species identified as B and H, etc. For the dissociation of an acid BH into its ions H^+ and B^-, $K = [H^+][B^-]/[HB]$. When the acid is half dissociated, so that $[B^-] = [HB]$, the pK is equal to the pH.

An "*electrolyte*" is any substance that can undergo partial or complete dissociation into ions, especially but not necessarily in solution, and which can therefore conduct an electrical current by the movement of the ions.

Practicing the Invention

The invention relates to non-enzymatic sensors, test kits and methods to quantitatively determine the physiologically relevant concentrations of sugars and related compounds in biological fluids. Generally, a test sample is contacted with a known concentration of a complexing agent, e.g., a meal complexing agent or a boronic acid compound of the formula:

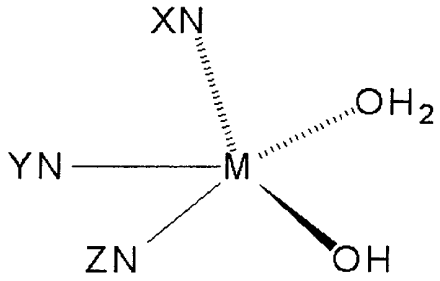


where R is hydrogen, or is a substituted or unsubstituted alkyl or aryl group.

When the sample contains a target molecule (e.g., a diol or sugar compound), the boronic acid compound forms a complex with the target molecule. More than one boronic acid molecule may bind to the target molecule. This results in a contemporaneous release of one or more protons (H^+) and a corresponding change in pH. The target molecule concentration can be determined by correlating the resulting pH change with the associated target molecule concentration, for example from a calibration plot. Because of its readability, reproducibility and accuracy, the invention is particularly suited for determining blood glucose concentrations within the biologically relevant range of importance to diabetic patients, *e.g.* 0 to 25 mM.

The change in proton concentration can be measured by its effect on the impedance in an electrical circuit that is formed between the electrode and the solution containing the complex. The flow of protons (and a corresponding flow of electrons) can be measured by an electrode, such as a metal electrode, in the sample, preferably in proximity to the interaction between the target sugar molecule and the boronic acid compound. In a liquid sample, such as an aqueous solution, the electrode can be placed in the sample. Preferably, the electrode and boronic acid compound can be brought together or combined to form a composite sensor. In one embodiment of the invention, the boronic acid compound is laminated to a metal electrode, preferably a gold electrode, and most preferably an interdigitated gold electrode.

A metal complexing agent may be substituted for a boronic acid complexing agent in the invention. Preferred complexing agents have the formula:



where M is any metal, preferably copper or iron; and

X, Y and Z are each hydrogen, carbon, or a functional group selected from styrene, methacrylate, acrylate, vinyl ether, vinyl acetate, trialkyloxysilane, dialkylchlorosilane,epoxy, other polymerizable groups, alkyl alkylhydroxyl or alkylamine groups having from 1-3 carbon atoms. One of the nitrogens in the formula can be replaced by oxygen or sulfur. X, Y and Z are preferably interconnected through bonded atoms, as shown by the curved lines in the formula above.

Exemplary metal complexing agents include:

Cu(TACN)	copper(II)-triazacyclononane;
Cu(1,4-dimethyl-TACN)	copper(II)-1,4-dimethyl-triazacyclononane;
Cu(EN)	copper(II)-ethylenediamine;
Cu(PDN)	copper(II)-propylenediamine;
Cu(IDA)	copper(II)-iminodiacetate;
Cu(DIEN)	copper(II)-diethylenetriamine;
Cu(N_2O-9-ane)	copper(II)-1-oxa-4,7-diazacyclononane; and
Cu(N_2S-9-ane)	copper(II)-1-thia-4,7-diazacyclononane.

Other exemplary metal complexing agents include (i) nitrogen-based bidentate ligands such as ethylenediamine and other diamines; (ii) nitrogen-based linear tridendate ligands; (iii) nitrogen-based linear tetradendate ligands; (iv) nitrogen-based tridentate macrocycles, such as triazacyclononane and polymerizable derivatives thereof; (v) nitrogen-based tridentate ligands similar to (iii) above, with at least one pendant arm able to further interact with a sugar molecule; (vi) nitrogen-based tetradentate macrocycle rings; and (vii) tridentate ring ligands.

Metal complexing agents that bind copper ions are most preferred, but any suitable metal may be used, including iron, lead, vanadium, mercury, nickel, cobalt, aluminum, uranium, calcium, barium, yttrium, and lathanum.

Preferably, metal ion complexes used in the invention should 1) hold the metal ion tightly; 2) allow at least two coordination sites to be or become available for binding to the target molecule. Further useful features are that the metal complexing agent is formed from a chelating ligand that may be modified to have a polymerizable group, for copolymerization; have functional groups appropriate for covalent attachment to a solid surface; and provide favorable interactions (*e.g.* electrostatic, hydrogen bonding, and hydrophobic) with the target molecule.

Metal complexing agents are preferably combined with or incorporated into a hydrogel, in a manner similar to that described for applications where the sensor composition comprises a boronic acid compound. Other suitable metal complexing agents are polymerizable, and are polymerized for example with styrene, methyl acrylate, methyl methacrylate, 2-hydroxyethyl methacrylate, 2-hydroxyethyl acrylate, acrylamide, vinyl ether, vinyl acetate, divinylbenzene, ethylene glycol dimethacrylate, ethylene glycol diacrylate, pentaerythritol dimethacrylate, pentaerythritol diacrylate, N,N'-methylenebisacrylamide, and trimethylpropane trimethacrylate.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 illustrates the decreases in pH of a test solution containing phenylboronic acid and *m*-nitrophenylboronic acid as a function of glucose concentration.

FIG. 2 is a plot displaying the changes in pH of a phenylboronic acid solution as a function of glucose concentration, alanine concentration, and lactic acid concentration.

FIG. 3 are plots of the pH change as a function of glucose concentration of a solution of phenylboronic acid, *p*-methylphenylboronic acid, *p*-methoxyphenylboronic acid, and *m*-nitrophenylboronic acid.

FIG. 4 is a plot of the decrease in pH of a 58.58 mM plasma solution of phenylboronic acid from its initial pH of 8.86, as a function of glucose concentration.

FIG. 5 illustrates the behavior of phenylboronic acid in whole blood as a function of glucose concentration.

FIG. 6 shows the decreases in pH of a solution of phenylboronic acid at 10 seconds, at 20 seconds, and at equilibrium as a function of glucose concentration.

FIG. 7 illustrates both the time and glucose concentration dependence of pH equilibration for phenyl boronic acid.

FIG. 8 illustrates the effect of temperature on the time required to reach pH equilibration.

FIG. 9 illustrates the difference in the induced pH change from solid phenylboronic acid and a 1:1 mixture of solid phenylboronic acid with its corresponding salt.

FIG. 10 is a plot of the decreases in pH of an imprinted polymer of 4-vinylphenylboronic acid as a function of glucose concentration.

FIG. 11 is a plot of the pH changes of a solution of phenylboronic acid as a function of dopamine concentration.

FIG. 12 is a schematic diagram of an exemplary laminated glucose sensor of the invention, comprising an electrode layer, a sensor composition layer, and a membrane layer.

FIG. 13A shows the time-dependence of the AC impedance of the electrode in a glucose sensor of the invention, plotted against incremental changes in glucose concentration of a test solution (Log Z). **FIG. 13B** shows incremental changes in the AC impedance of the electrode at a steady state with increasing glucose concentration in a test solution. **FIG. 13C** shows the time-dependence of the first derivative (with respect to time) of the AC impedance of the electrode, with incremental increases in glucose concentration in the test solution. **FIG. 13D** shows that the maximum rate of change in the AC impedance of the electrode depends upon the concentration of glucose in the test solution. **FIG. 13E** is a replot of **FIG. 13A** in conductance units. **FIG. 13F** is a replot of **FIG. 13B** in conductance units.

FIG. 14 is a comparison of incremental changes in AC impedance of a glucose sensor electrode at steady state, with changing glucose concentrations in a test solution, for (a) a laminated sensor made with a boronic acid sensor composition affixed to a metal electrode, and encapsulated by a bipolar-ED-membrane; and (b) the same sensor without the membrane.

FIG. 15 is a plot of the change in impedance in a laminated glucose sensor, when various amounts of glucose are added to a sample of porcine plasma that originally contained about 5 mM glucose.

FIG. 16 is a plot of reduced hydrogen ion concentration (Y_H) as a function of glucose concentration in the test solution (X_G) for various values of the ratio of the ionization constants of glucoboronic acid and boronic acid ($K_{A(BGH)}/K_{A(BH)}$). This plot illustrates the dependence of sensor response on the ratio of the ionization constants of phenylglucoboronic acid and phenylboronic acid.

FIG. 17 is a plot of reduced hydrogen ion concentration (Y_H) as a function of reduced glucose concentration (X_G), in a sensor matrix containing boronic acid partially deprotonated by

neutralization with NaOH, for various degrees of neutralization of boronic acid ($[BH_o]/[Na^+_o]$). In this plot, $K_{A(BGH)}/K_{A(BH)} = 690$.

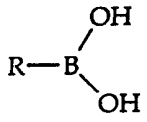
FIG. 18 is a plot of relative hydrogen ion concentration (R) as a function of reduced glucose concentration (X_G), in a sensor matrix containing boronic acid partially deprotonated by neutralization with NaOH, for various degrees of neutralization of boronic acid ($[BH_o]/[Na^+_o]$). In this plot, $K_{A(BGH)}/K_{A(BH)} = 690$.

FIG. 19 is a plot of relative hydrogen ion concentration (R) as a function of reduced glucose concentration (X_G), in a sensor matrix containing partially NaOH-deprotonated boronic acid, for varying values of the ratio of the ionization constant of glucoboronic acid to that of boronic acid ($K_{A(BGH)}/K_{A(BH)}$). In this plot, $[BH_o]/[Na^+_o] = 1000$.

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to non-enzymatic sensors, devices and methods for measuring levels of any target molecule or analyte, such as carbohydrate or diols, especially cis-diols, in a sample. More particularly, the invention relates to the use of compounds, polymers and copolymers having one or more metal complexing agents or boronic acid groups ($-B(OH)_2$) for quantitatively measuring sugar concentrations in biological fluids, especially glucose concentrations in a liquid (*e.g.* aqueous) sample, such as interstitial fluid, blood. Biologically or physiologically relevant blood glucose concentrations are glucose concentrations between about 0 mM and about 25 mM (about 0 mg/dL and about 450 mg/dL). In a preferred embodiment, quantitative determination of glucose includes the ability to measure glucose levels of at least 5 mM (about 90 mg/dL), and more preferably between about 1.0 mM and about 3 mM (between about 25 mg/dL and about 50 mg/dL). Most preferably, glucose determinations can be made with an accuracy of about ± 0.5 mM.

The boronic acids of the present invention are generally of the formula



wherein R is selected from a group consisting of hydrogen, unsubstituted alkyl, substituted alkyl, unsubstituted aryl, and substituted aryl. The term "alkyl" refers to a non-aromatic hydrocarbon compound. Illustrative examples include but are not limited to alkanes, alkenes, and alkynes.

The term "aryl" refers to aromatic compounds and is intended to include both aromatic hydrocarbons and aromatic heterocycles (*i.e.* furan, pyrrole and thiophene). Illustrative examples of both alkyl and aryl substitutions include but are not limited to C_1-C_5 alkyl, C_1-C_5 alkoxy, and phenyl. In addition, R may include one or more functional groups selected from the group consisting of hydroxyl, thiol, thioether, ketone, aldehyde, epoxy, ester, ether, amine, imine, amide, nitro, carboxylic acid, disulfide, carbonate, isocyanate, carbodiimide, carboalkoxy, carbamate, halogen, and the same or another boronic acid.

In preferred embodiments, R is unsubstituted phenyl or a substituted phenyl which includes C_1-C_5 alkyl or one or more functional groups selected from the group consisting of hydroxyl, thiol, thioether, ketone, aldehyde, ester, ether, amine, imine, amide, nitro, carboxylic acid, disulfide, carbonate, isocyanate, carbodiimide, carboalkoxy, carbamate, and halogen. Use of phenylboronic acid, *p*-methoxyphenylboronic acid, *p*-methylphenylboronic acid, and *m*-nitrophenylboronic acid, and their polymerizable derivatives, is most preferred in the practice of the invention.

A subset of the boronic acids of the present invention are polymerizable boronic acids in which R includes at least one polymerizable group. Aromatic boronic acid polymers are preferred. Illustrative examples of polymerizable groups include but are not limited to styrene, methacrylate, acrylate, vinyl, vinyl ether, vinyl acetate, trialkyoxysilane, dialkylchlorosilane, epoxy, alkylhydroxyl, and alkylamine. Functional groups such as those described above may also be incorporated into the polymers. In embodiments where the polymer is immobilized onto an electrode, polymers that are soluble in organic solvents and are insoluble in aqueous solutions (water) are preferred. Examples of boronic acid polymers include, without limitation:

poly(vinyl phenyl boronic acid);
poly(vinyl 3-nitrophenylboronic acid);
poly(vinyl 3-methoxyphenylboronic acid);
poly(vinyl 3-carboxyphenylboronic acid);
poly(vinyl 5-bromophenylboronic acid);
poly(vinyl 3-methylphenylboronic acid);
poly(vinyl 3,5-dichlorophenylboronic acid); and
their polymerizable derivatives.

The species to be complexed by this polymer (glucose or another diol) is predominantly water-soluble, and must be accessible to the boronic acid group of the polymer. Thus, the polymer must

be sufficiently hydrophilic to allow free access to the substrate glucose molecule. The polymer can be made more hydrophilic by incorporating hydrophilic monomers and polymers.

Boronic acid compounds, according to the invention, are used to measure the concentration of various analytes or target molecules, particularly cis-diols, that release a proton (H^+) or a hydroxyl anion (OH^-) in the presence of the boronic acid compound, *e.g.* by forming a complex with the boronic acid compound. Target molecules for boronic acid sensor compositions include but are not limited to diols, cis-diols, saccharides, and sugars (especially glucose). When a metal complexing agent is used, the target molecules include carbohydrates; glycerol; dopamine; catechols; ascorbic acid; 1,4, anhydroerythritol; ethleneglycol; and 3-hydroxy-L-tyrosine. The concentration of protons or hydroxyl anions can easily be measured, and can serve as a measure of the target molecule concentration when the concentration of the proton or hydroxyl anion released is a function of or is proportional to the concentration of the target molecule.

The mechanism of the complexation reaction between sugar and boronic acid compound is not well understood, nor is the structure of the complex. Reports have speculated that both 1:1 and 1:2 complexes of target molecule to boronic acid exist, in which the equilibrium between the two complexes is dependent upon pH and the relative concentrations of boronic acid and target molecules. At least one laboratory has found evidence of a positive binding cooperation resulting in the possibility of a 1:4 target molecule to boronic acid complex. *See* D'Silva and Green, J. Chem. Soc. Chem. Commun. 227-229 (1991). According to the invention, however, it is not necessary to know or understand the chemistry or structure of the interaction, nor the particular ratio or amount of ion release for each target molecule that interacts with the sensor composition. For purposes of the invention, it is sufficient that the interaction produces a detectable or measurable change in ion concentration that is relative to and can be correlated with the concentration of target molecule.

The conditions which are suitable, preferred, or optimal for monitoring proton release or pH change as a measure of target molecule concentration are readily determined experimentally using conventional techniques. These conditions depend on the particular target molecule and complexing agent, and on the dynamics of the interaction between them by which ions are released and, it is believed, a complex is formed. Using empirical determinations from known amounts of target molecule and complexing agent, a reaction system can readily be optimized and

calibrated, so that observed changes in conductance, impedance, pH, or other detection method, can serve as a quantitative marker for the concentration of target molecule in a test sample. Although the specific parameters of such experiments will necessarily be context dependent, the general protocol is routine, will be similar in all instances, and can readily be employed by the skilled artisan without undue experimentation. For convenience in describing these techniques (which are readily adaptable), the invention is described primarily with reference to a preferred embodiment: measuring blood glucose levels.

Normal blood glucose levels, for example in individuals who do not suffer from diabetes, is within a relatively narrow range of about 4 to 5 mM. Diabetics can experience fluctuating blood glucose levels within a range of about 3-25 mM. Thus, the relevant concentration range for measuring blood glucose in the context of diabetes is between about 0 mM and about 25 mM. Because of insufficient insulin production, diabetic patients must routinely monitor their blood glucose levels and self-administer insulin when levels exceed the normal range, for example, when glucose concentration exceeds 5 mM.

Too much or too little glucose in the blood (hyperglycemia and hypoglycemia) can cause serious physiological problems, including retinopathy, neuropathy, heart disease, kidney failure, coma and death. Insulin is a protein or hormone produced by the body to regulate the amount of glucose in the blood. It has long been known that individuals with an insulin deficiency may have difficulty controlling blood sugar levels, with potentially serious and even life-threatening consequences. It has also long been known that various forms of insulin can be administered to the blood stream artificially, to supplement or ameliorate an insulin deficiency and better control glucose levels. However, it is difficult to maintain blood glucose within the relatively narrow normal range by administering insulin, particularly if the amount or concentration of glucose in the blood is unknown. Blood glucose levels fluctuate in response to food, metabolism, and other factors, and the dosage and timing of insulin therapy is difficult to precisely regulate, so that optimum blood glucose levels are maintained.

In view of the normal and diabetic ranges of glucose concentration, detection of at least 5 mM, more preferably between about 1.0 mM and 3 mM, and preferably with an accuracy of ± 0.5 mM, is needed in order to differentiate normal glucose concentrations from concentrations that require an insulin injection. In addition, while blood glucose levels higher than about 25 mM are possible, it is generally less important to quantitatively measure such high concentrations

because such a patient would be extremely ill. This scenario would not likely occur in an out-patient context, and in any case the primary goal of monitoring glucose levels would be to detect an increase in blood glucose, and appropriately intervene, before so a high a concentration in the blood can occur.

Thus, a boronic acid compound or metal complex for use in a glucose sensor is selected to provide a reaction with glucose, and a corresponding release of protons, that is measurable at glucose concentrations of from about 0 to 25 mM, with an accuracy of about ±5 mM, preferably from about ±1.0 mM to about ±3 mM, and most preferably ± 0.5 mM. This can easily be determined empirically, using known amounts or concentrations of glucose and boronic acid compounds.

Another factor in selecting a particular complexing agent (*e.g.* boronic acid compound or metal complex) is the desired initial pH, which is typically dependent on the pH of the sample and on the compound's acid dissociation constant or pKa. Changes in local pH from interaction between a complexing agent and target molecule are easier to measure at pH values where the sample has little or minimal buffering capacity. Similarly, the sensor tends to be more sensitive at pH values where the complexing agent strongly interacts with the target molecule. The high buffering capacity of biological fluids at physiological pH can reduce the reproducibility or responsiveness of a complexing agent and sensor, for example by quenching ions produced by interaction with the target molecule. The buffering capacity of biological fluids typically reaches a minimum near a pH of about 8. Thus, the initial pH of a sample of biological fluid can be adjusted to a pH of 8 or higher, to minimize buffering effects.

To minimize the buffering capability of the boronic acid itself, which can occur when pH = pKa, it is generally preferred to adjust the initial pH so that it is at least 0.5 more or less than the pKa (pH $\leq$ pKa - 0.5 or pH $\geq$ pKa + 0.5). In more preferred embodiments, the initial pH is between 1.0 and 1.5 more or less than the pKa (pH $\leq$ pKa - 1.0 to 1.5 or pH $\geq$ pKa + 1.0 to 1.5). In further preferred embodiments, the pKa of the boronic acid compound is more than the pH of a sample to be tested for a target molecule, preferrably 0.5 to 1.5 more. This can be done by selecting boronic acid compounds with suitable pKa values for the test samples, adjusting the initial pH of the samples before testing, or a combination thereof. For example, when testing biological samples having a physiological pH (*e.g.* 6.8-7.5, esp. 7.4), a boronic acid compound can be selected with a pKa above 7.8, preferably above 8 or 9, and more preferably in the range

of about 8.5 to 9.5. Similarly, the initial pH can be adjusted to about 8 to 8.5, and the pKa of the boronic acid compound can be selected to be about 0.5 to 1.5 higher, for example about 9 to 10.

The use of a bipolar membrane interposed between the sample and the sensor composition serves to reduce or eliminate the dependence of the senor response on the buffering capacity of the sample. When the bipolar membrane is used, the ions released upon glucose complexation with the complexing agent are retained in the sensor matrix and there is no quenching of the signal due to buffering from the sample itself. Furthermore it is possible to maintain the sensor composition or matrix at a pH that is different from the pH of the sample. This allows the sensor to operate at the pH that gives the best signal, which may not necessarily be the pH of the sample.

In a preferred embodiment, the bipolar ion exchange membrane can overlay a hydrogel sensor composition without disruptive conductimetric interference when the distance of the membrane from the electrodes is great enough, and the spacing of electrodes (*e.g.* the interdigital spacing) is small enough, so that the primary current-pathway is the gap between electrodes (*e.g.* between adjacent interdigitating electrodes.

The sensor can be refreshed or equilibrated to minimize buffering or other disadvantageous effects, such as decreased accuracy or longer response time. This can be done by washing the sensor in a salt solution (*e.g.* sodium chloride) and by equilibrating the sensor in a standard solution of known target molecule concentration, at a controlled pH and temperature.

For convenience and simplicity, it is generally preferred that the boronic acid compound be commercially available or easily prepared. If modifications are made (as with polymerizable boronic acids), it is preferred that the synthesis be simple (*e.g.* three steps or less) with yields greater than 50% of the initial starting material.

Illustrative examples of suitable boronic acids and their pKa's include:

phenylboronic acid,	pKa ≈ 8.9;
p-methoxyphenylboronic acid,	pKa ≈ 9.3;
p-methylphenylboronic acid,	pKa ≈ 9.3;
m-nitrophenylboronic acid,	pKa ≈ 7.4.

However, any boronic acid and initial pH may be used that provides a quantifiable correlation between a measured change in pH and glucose concentration. Any other detection method may also be used, such as fluorimetry, colorimetry, light scattering, and other ways of measuring conductance, pH or ion concentration. For example, in another variation static pH titration may

be used to determine target molecule concentration. For example, if the complexation reaction results in a decrease in pH from initial levels, instead of measuring the pH change, an amount of a suitable base (*i.e.* NaOH) is added to maintain the initial pH. In other words, the amount of base necessary to maintain pH is used as a surrogate marker for pH change, and to determine the concentration of the target molecule.

A correlation between the change in pH and target molecule (*e.g.* sugar) concentration can be empirically determined. **FIG. 1** illustrates such an experiment for glucose in the presence of phenylboronic acid and *m*-nitrophenylboronic acid. Because of the correlation (*e.g.* one-to-one) between pH change and glucose concentration in the physiologically relevant range, the magnitude of the pH change may be used as a surrogate marker for glucose concentration.

For example, the concentration of glucose in a sample may be determined by using **FIG. 1** as a calibration plot. First, the pH of the sample is adjusted to pH 7.4 by adding an acid, *e.g.* sodium hydroixide (NaOH) or hydrochloric acid (HCl) depending on the sample's initial pH. The sample is then contacted with the same concentration of boronic acid compound (*e.g.* phenylboronic acid or *m*-nitro-phenylboronic acid) that was used in the calibration plot of **FIG. 1**. The pH of the resulting solution is then measured, and the glucose concentration is determined by correlating the resulting pH change with its associated glucose concentration from the calibration plot.

Additional experiments are conducted to determine whether a quantifiable correlation also exists between pH change and glucose concentration in a more complex sample, such as plasma or blood, which contains many ingredients in addition to glucose and water. For example, because plasma and blood also contain a number of additional components, it is possible that one or more of these may also interact with the boronic acid compound, and thus may interfere with the accuracy of a blood glucose measurement.

To test this possibility, the interaction of alanine and lactic acid with phenylboronic acid was measured. Because proteins and free amino acids are expected to be a significant component of plasma and blood, alanine was tested as a representative amino acid, the constitutive component of proteins. As illustrated by **FIG. 2**, addition of alanine has no effect on the pH of the sample. Increasing concentrations of lactic acid resulted in a noticeable but very slight and generally negligible increase in pH, except at very high concentrations of lactic acid. Consequently, this small side effect can be ignored when determining blood glucose

concentrations. Alternatively, when a higher sensitivity glucose measurement is desired, the glucose correlation plots may be adjusted to include this effect.

In view of the alanine and lactic acid experiments, explicit correlations between pH change and glucose concentrations were prepared for both plasma and blood for several boronic acid compounds. Illustrative results are shown by **FIGS. 3-5**, which demonstrate that the resulting pH change is substantially linear with increasing glucose concentration in the biologically relevant range.

The relationship between pH change and glucose concentration is affected by several factors. For example, **FIG. 6** illustrates that both non-equilibrium (10 seconds and 20 seconds) and equilibrium time points displayed a linear relationship between pH change and glucose concentration. **FIGS. 7 and 8** show the effect of glucose concentration and temperature respectively on the amount of time the system takes to reach its equilibrium pH. Because both the kinetics and the magnitude of the pH change is dependent upon environmental conditions, to the extent possible, test sample conditions should mirror the correlation plot conditions. Note also that conditions can be manipulated to achieve certain desired results. For example, a more rapid equilibrium can generally be achieved at higher temperatures, and higher output signals from the sensor can generally be obtained by using higher concentrations of complexing agent.

The boronic acid compounds of the present invention may include one or more polymerizable groups, which can be exploited to form boronic acid polymers. Illustrative examples of such groups include but are not limited to:

styrene,
methyl methacrylate,
2-hydroxyethyl methacrylate,
2-hydroxyethyl acrylate,
methyl acrylate,
acrylamide,
vinyl ether,
vinyl acetate,
divinylbenzene,
ethylene glycol dimethacrylate,
ethylene glycol diacrylate,
pentaerythritol dimethacrylate,
pentaerythritol diacrylate,
N,N'-methylenebisacrylamide,
N,N'-ethylenebisacrylamide,
N,N'-(1,2-dihydroxyethylene)bis-acrylamide, and

trimethylolpropane trimethacrylate.

Moreover, one or more of the above compounds may also be used as co-monomers for making boronic acid polymers.

The boronic acid compound can be in solid or gel form, or in salt form. For example, **FIG. 9** shows the induced pH change in response to glucose or solid phenylboronic acid and a 1:1 mixture of solid phenylboronic acid with its corresponding salt. The polymerizable boronic acid may be polymerized using any conventional technique.

In one embodiment, the boronic acid polymer is imprinted with a template molecule (the target molecule or its analog) to form selective binding sites within the polymer. Imprinting techniques have been previously described by Wulff, G., Molecular Recognition in Polymers Prepared by Imprinting with Templates, *"Polymeric Reagents and catalysts* (W. T. Ford ed.)", ACS Symposium Series 308, American Chemical Society, Washington, D.C., 186-230 (1986); Wulff, G. Biorecognition in Molecularly Imprinted Polymers: Concept, Chemistry, and Applications, in *"Molecular Interactions in Bioseparations* (T. Ngo ed.)", Plenum Press: New York, 363-381 (1993); and Wulff, G., Schauhoff, S., "Racemic Resolution of Free Sugars with Macroporous Polymers Prepared by Molecular Imprinting.

For example, for bulk polymerization it is preferred that between about 5 and 10 weight percent of polymerizable boronic acid is reacted with between about 90 and 95% of another polymerizable monomer (*e.g.* a cross-linking monomer) in the presence of about 1% of a free radical initiator (*i.e.* azo-bis(isobutyronitrile) ("AIBN"). The polymerization reaction preferably occurs between about 60 °C and about 70 °C in a mixed aqueous and organic solvent for approximately 24 hours. The solvent, temperature, means of polymerization (*e.g.* free radical initiation, gamma-radiation, etc.) can be varied in known ways to obtain polymers having desired or optimized chemical and physical characteristics, such as porosity, stability, and hydrophilicity. The resulting cross-linked polymer is cut into pieces or ground into a powder and washed thoroughly.

Briefly, the imprinting technique involves the following. A polymerizable boronic acid compound is allowed to bind a template compound such as glucose analog, methyl-α-D-glucopyranoside. This boronic acid-template compound complex is then co-polymerized with a suitable monomer or cross-linking agent to form a porous polymer structure. The template compounds are then removed, using conventional techniques such as exhaustive washing with

acidic solution. This leaves binding sites that are especially adapted for binding the target molecule. **FIG. 10** shown the decreases in pH of imprinted 4-vinylphenylboronic acid as a function of glucose concentration.

Both non-imprinted and imprinted boronic acid polymers may be prepared in any form (*i.e.* powders, beads, rods, membranes, coatings) or optionally, may be incorporated into a variety of support or matrix structures. Illustrative examples of support or matrix structures include but are not limited to silicon, glass, quartz, ceramics, other polymers (*e.g.* hydrogels), and zeolites. In addition, if desired, pH-sensitive indicators such as fluorescent probes may also be incorporated into these polymers for detecting glucose using optical methods.

Although the invention has been described mainly in terms of measuring blood glucose levels, the same techniques can be readily adapted to a variety of contexts. For example, as illustrated by **FIG. 11**, the invention may be used to measure dopamine concentrations. In this context (initial pH = 9.38), the complexation of phenylboronic acid with dopamine as the target results in a corresponding release of hydroxide anion.

In a preferred embodiment of the invention, a sensor is provided, in which a metal electrode is laminated to a sensor composition comprising a blend of a boronic acid polymer and a hydrogel. *See e.g.* **FIG. 12**. Suitable boronic acid polymers are identified above. A preferred polymer is poly(vinyl phenyl boronic acid). Because the polymer is immobilized onto an electrode, polymers that are soluble in organic solvents and are insoluble in aqueous solutions (water) are preferred. The sensor layer typically has from about 25-75% boronic acid polymer, and the remainder is hydrogel plus other optional components such as stabilizers and preservatives.

Any suitable hydrogel may be used. A non-ionic or non-ionogenic hydrogel is particularly preferred. Examples include poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether). A preferred hydrogel is poly(2-hydroxyethyl methacrylate). One or more polymers can be used in combination with one or more hydrogels. Ionic or ionogenic gels should generally be avoided, because such gels may alter or contribute to the conductivity of the electrode. An important consideration in providing an accurate and sensitive electrode and sensor, is that the primary (if

not sole) source of conductive ions in the electrode and sensor matrix should be the ions formed by interaction between the complexing agent and the glucose or other target molecule.

In a more preferred embodiment, a thin bipolar membrane is layered over the sensor layer, or may encapsulate the sensor composition and electrode. Suitable membranes include polystyrene and polysulfone-based membranes. A preferred membrane is comprised of a layer of crosslinked sodium polystyrene sulfonate bonded to a layer of crosslinked poly(vinyl benzyl trimethyl ammonium chloride), provided by Aqualytics Company, Warren, NJ.

During use, the sensor is exposed to or immersed in a sample solution, and the impedance of the electrode is measured. For example, the electrode can be connected to an electrochemical workstation, and its impedance is measured at high frequency. The sugar (*e.g.* glucose) level in the sample is determined by correlating the measured impedance to previously established standards, obtained for example by measuring the impedance in solutions containing known concentrations of glucose, or other target molecule.

The laminated sensor, comprising a sensing layer and electrode, can be represented as an electrical circuit when placed in a glucose-containing solution. The circuit, also called an "equivalent" electrochemical circuit, comprises three key components: (1) faradaic impedance (Z_f); (2) resistance between working electrodes (R); and (3) double layer capacitance (C_d). In preferred embodiments the difference in electrical potential between working electrodes is very small (about 5 mV). Consequently, oxidation and reduction reactions which might otherwise interfere with glucose-dependent impedance measurements do not appreciably occur on the electrode.

When impedance is measured with a sufficiently low applied AC voltage (where electrolysis is avoided) and at a moderately high AC frequency (where electrode polarization and dipole orientation is undetectable), the measured impedance is primarily the ohmic resistance of the matrix, determined by the concentration, mobility and charge of the ions in the matrix without any meaningful contribution from other variables. Suitable voltages are typically between 0.05 and 1 volts. Suitable frequencies are typically above 1.0 KHz and below 10 KHz. Glucose molecules in a sample bind to the boronic acid polymer, such as poly(vinyl phenyl boronic acid), and protons are released. This increases the ionic concentration of protons in the polymer matrix. The amount or concentration of protons is proportional to the amount of glucose in the sample, and the resistance and consequently the impedance of the electrode is correspondingly decreased.

As a result, the measured change in impedance is a function of, and is substantially proportional to the concentration of glucose in the sample.

Sensor compounds (electrode and sensor composition) can be encapsulated by a bipolar membrane. Glucose molecules, which are neutral, can diffuse through the membrane. Charged molecules do not cross the membrane, and do not interfere with the interaction of glucose and boronic acid polymer at the electrode. For example, the presence of charged molecules which might alter the ionic concentration of protons and influence the impedance measurement are shielded from the electrode by the membrane. The use of a highly charged membrane significantly retards the diffusion of ions (*i.e.* ions formed by the interaction of glucose and boronic acid polymer) away from the electrode and out into the sample. Neutral species such as glucose and water can freely pass the membrane and reach the sensor layer and electrode. A bipolar membrane also serves to exclude other ionic or ionogenic compounds present in biological samples, and which may interfere with the evaluation of glucose concentration. In addition, the membrane experiences limited hydration, and consequently is impermeable to ionic and non-ionic water-soluble macromolecules (*e.g.* of 1000 Daltons and greater) that are found in blood or other biological samples, and which might affect the accuracy of the sensor response. For example, inherent buffering effects caused by organic anions and cations in biological samples can weaken signals based on hydrogen (proton) or hydroxide ion concentrations. However, these species do not readily penetrate the bipolar membrane. Thus, the use of a membrane permits more sensitive and accurate determinations of glucose concentration when desired.

Thus, without the membrane, ions in the target solution can enter the hydrogel layer and alter the conductance of that layer, and/or react with hydrogen ions liberated by glucose complexation, which can produce inaccurate impedance measurements. Also, hydrogen ions liberated in the hydrogel layer by glucose complexation, or other ions initially present in the layer, can exchange with

similarly charged ions in the target solution and may also alter the impedance. The bipolar membrane prevents such ion intrusion or interchange, and assures that the impedance is uniquely correlated with the glucose concentration in the hydrogel layer.

Membranes of this type are known. K.N. Mani, *et al.*, *Desalination*, 68: 149 (1988); H. Strathmann, *et al.*, *J. Membrane Sci.* **125**: 123 (1997); R.J. Simons, *Membrane Sci.*, **78**: 13 (1993).

Suitable bipolar membranes include polystyrene and polysulfone-based membranes. A preferred membrane is comprised of a layer of crosslinked sodium polystyrene sulfonate bonded to a layer of crosslinked poly(vinyl benzyl trimethyl ammonium chloride), provided by Aqualytics Company, Warren, NJ. Bipolar membranes may also be obtained from Tokuyama Corp., Yamaguchi Japan and FumaTech GmbH, St. Ingbert, Germany.

The component laminate-layers of the bipolar membrane should preferably be comprised of crosslinked linear polymers or copolymers of high thermal and chemical stability which contain, or bear functional groups which can be subsequently chemically coupled to, ionogenic compounds derived from strong acids or strong bases to yield polyelectrolytes of high charge-density or ion-exchange capacity and water-affinity.

Cation exchange resins suitable for the cation exchange layer of such a laminate include, but are not limited to crosslinked poly(styrene) or poly(vinyltoluene) produced by copolymerization with 1-10 % by weight of di(vinylbenzene), subsequently reacted with chlorosulfonic acid to yield a water-insoluble but water-swellable aryl sulfonic acid containing between 0.5 and 1.0 mol of sulfonate group per mer-mol of polymer; or any benzenoid monomer capable of polymerization or copolymerization to yield a crosslinked polymer of high aryl content, amenable to functionalization with strongly acidic groups. Aromatic polysulfones, aromatic polyethers and polycarbonates, and poly(para-phenylene) are examples of such materials. Other suitable materials for the cation exchange layer include copolymers of perfluoroalkylvinyl monomers subsequently sulfonated with reagents such as chlorosulfonic acid or sulfonyl chloride, subsequently subjected to aqueous hydrolysis to yield a water-swellable sulfonate cation exchanger of high charge density or cation exchange capacity. Such polymers do not require crosslinking to prevent water-dissolution, but undergo limited swelling in water to yield a structure containing between 1.0 and 8.0 gmols of exchangeable cation per liter of polymer.

Anion exchange resins suitable for the anion exchange layer of such a laminate include, but are not limited to crosslinked poly(vinylbenzyl chloride), poly(vinyl chloride), or other halide-containing vinyl monomer, produced by copolymerization with 1-10 % by weight of di(vinylbenzene) or butadiene, and subsequently reacted with trimethyl amine, pyridine, or other tertiary amine to yield a water-insoluble but water-swellable alkyl or aralkyl quaternary ammonium containing between 0.5 and 1.0 mol of quaternary ammonium group per mer-mol of

polymer; or any benzenoid monomer capable of polymerization or copolymerization to yield a crosslinked polymer of high aryl content, amenable to functionalization with quaternary ammonium groups. Halogenated aromatic polysulfones, aromatic polyethers and polycarbonates, and poly(para-phenylene) are examples of such materials. Other suitable materials for the cation exchange layer include copolymers of perfluoroalkylvinyl and/or perfluorochloroalkyl vinyl monomers subsequently quaternized with tertiary alkyl or alkaryl amines, to yield a water-swellable quaternary ammonium anion exchanger of high charge density or anion exchange capacity. Such polymers do not require crosslinking to prevent water-dissolution, but undergo limited swelling in water to yield a structure containing between 1.0 and 8.0 gmols of exchangeable anion per liter of polymer.

Both cation- and anion-exchangers as described above can be conveniently fabricated in thin membrane form by casting a thin liquid film of mixed monomers and an appropriate polymerization catalyst on a polished metal or glass plate, and allowing polymerization to take place. When polymerization is complete, the resulting plate containing the polymer film is immersed in a liquid bath containing the ionogenic reagent of interest, and the reaction between the polymer and reagent allowed to proceed to the desired extent. The plate and adherent film is then immersed in water (or aqueous acid or base, if so desired) to remove unreacted reagent and water-soluble byproducts, conversion to the desired exchangeable cation or anion, and to allow hydration and swelling of the film. The film usually detaches from the plate in this process, and can be washed further in water to remove residual soluble components. The membrane is then sealed in a hermetic package to prevent water-loss until ready for use. Other means for producing thin films of polymeric materials can be employed, as will be recognized by those skilled in the art.

Lamination of the cation and anion exchange membranes to yield the desired bipolar membrane typically requires special care and proper selection of bonding agents, to insure that the laminate has the desired permeability to nonionic microsolutes and virtually total impermeability to ions. This can be done by providing an ultrathin bonding layer of high water sorptivity and strong

adhesion to both adjacent membrane layers. Water-soluble polymeric adhesives capable of crosslinkage to yield high-strength hydrogels are particularly attractive for this purpose. These compounds include but are not limited to poly(vinyl alcohol) crosslinked by reaction with

glyoxal; poly(acrylamide) crosslinked by reaction with formaldehyde; or isocyanate-terminated polyethylene glycol of moderate molecular weight. Such adhesive combinations are applied as concentrated aqueous solutions to one of the two component-membranes as a thin layer, the second membrane applied to the coated surface via squeegee to assure close contact and elimination of air bubbles, and the laminate held under moderate pressure until the adhesive curing process is complete. Other means for carrying out such a bonding operation, and other adhesive materials meeting requirements specified above, will be well-known to those skilled in the art.

Although the present invention is described with reference to preferred embodiments, these embodiments are for purposes of illustration. It will be appreciated by persons of ordinary skill in the art that these descriptions, embodiments and examples do not limit the scope of the invention or the appended claims.

EXAMPLE 1

Change in pH as a function of target molecule concentration

A general protocol for determining pH change as a function of target molecule concentration is as follows. For convenience, phenylboronic acid and glucose are be used to illustrate this method. 1.5 mL of a 10 mM aqueous phenylboronic acid solution is placed in a titrating vessel (Metrohm, No. 719 S Titrino) at 25 °C. The vessel is equipped with a stirring bar, temperature control bath and pH meter. The pH of the phenylboronic acid solution is adjusted to 7.4 using 0.1 N NaOH or 0.1 N HCl depending on the initial pH. A stock solution of 0.5 M glucose (at pH 7.4) is prepared. 10 uL of the stock glucose solution is added to the phenylboronic acid solution. When the pH becomes stable, the final pH is noted.

At this point, another 10 uL aliquot of the glucose solution is added to the phenylboronic solution and the resulting pH is noted. This procedure is continued until a total of 200 uL of the glucose solution has been added to the initial phenylboronic acid solution. **FIG. 1** is a plot of the pH change versus glucose concentration for both phenylboronic acid and *m*-nitrophenylboronic acid in this experiment. Note that the initial pH of both phenylboronic acid curves started at pH 7.4.

Other boronic acid derivatives may be substituted for phenylboronic acid and other cis-diols may be substituted for glucose in this protocol.

The concentration of target molecule in a sample of unknown concentration can be determined by measuring the pH change when the sample is contacted with the boronic acid under similar conditions, and comparing the pH change with a curve prepared as described above.

EXAMPLE 2

Interaction of lactic acid and alanine with phenylboronic acid

Lactic Acid. The effect of lactic acid ($CH_3CHOHCOOH$) on the pH of a solution of phenylboronic acid was investigated. 1.5 mL of 10 mM aqueous phenylboronic acid solution was placed in a titrating vessel at 25 °C. The pH of this solution was adjusted to pH = 7.4 using either 0.1 N NaOH or 0.1 N HCl. A stock solution of 0.5 M aqueous solution of lactic acid solution (pH = 7.4) was prepared. 10 uL of the lactic acid solution was added to the phenylboronic acid solution and the pH was noted after the solution equilibrated. 10 uL aliquots of the stock lactic acid solutions were added and the pH noted until a total of 200 uL of lactic acid had been added.

Alanine. The effect of alanine on the initial pH of a solution of phenylboronic acid was also investigated. The procedure used was identical to one used for lactic acid except that a 0.5 M solution of alanine was prepared (pH = 7.4) and 10 uL aliquots of this alanine solution was added to the initial 1.5 mL of 10 mM aqueous phenylboronic acid (pH = 7.4).

FIG. 2 overlays the results of the phenylboronic acid interaction with glucose, alanine, and lactic acid respectively. As shown, lactic acid had little or no effect on pH, except at very high concentrations, and alanine had no observable effect. These results indicate that blood glucose levels can be accurately and reliably determined, at physiologically significant concentrations and sensitivities, without disruptive interference from these other blood components. Similar experiments can readily be performed, if desired, using other target molecules, boronic acid compounds, and potentially interfering compounds.

EXAMPLE 3

Glucose measurements in porcine plasma

1.5 ml of porcine plasma was placed in the titration vessel at 25 °C and 28 mmoles of solid phenylboronic acid was added and the pH of this solution was adjusted to 7.6 with 0.1 N NaOH. A stock solution of 0.5 M glucose solution made from porcine plasma was prepared and the pH of this solution was also adjusted to 7.6. 20 uL aliquots of the stock glucose solution

(Example 1) was added and the pH was noted after the resulting solution reached equilibrium. This procedure was repeated until a total of 100 uL of stock glucose solution was added.

This procedure was repeated with other boronic acid compounds, namely *p*-methylphenylboronic acid (initial plasma pH adjusted to 8.5); *p*-methoxyphenylboronic acid (adjusted to pH=8.5); and *m*-nitropheynlboronic acid (pH=6.9). Plots of the pH change of porcine plasma for all four of the boronic acid derivatives as a function of glucose concentration is shown in **FIG. 3**.

EXAMPLE 4

Monitoring Blood Glucose Levels in the Physiological Range

To five 1.5 mL samples of porcine plasma, known volumes (0 uL, 15 uL, 30 uL, 60 uL, and 120 uL) of 0.5 M aqueous glucose solution were added, resulting in plasma samples having different glucose concentrations (0 mM, 4.79 mM, 9.43 mM, 18.52 mM, and 35.71 mM) respectively. The pH of each of the samples was adjusted to 8.6 using 0.1 N NaOH. 15.4 mg (68 mmol) of solid *p*-methoxylphenylboronic acid was added to each plasma sample and the change in pH was noted. **FIG. 4** shows that the resulting decrease in pH is linear with respect to glucose concentration in the biologically relevant range.

EXAMPLE 5

Glucose Measurements in Whole Blood

A 38.85 mm solution of phenylboronic acid was prepared in whole porcine blood. The pH of this phenylboronic acid solution was adjusted to 7.50 using 0.1 N NaOH. 1.5 ml of this solution was placed in the titrating vessel and known amounts of 0.5 M aqueous solution glucose (pH 7.5) were added. The resulting changes in pH were measured. **FIG. 5** shows that the decreases in pH induced from the complexation of boronic acid with glucose is substantially linear with increasing glucose concentration within the biologically relevant range.

EXAMPLE 6

Response Time of Glucose Concentration Measurements

A 58.58 mM solution of phenylboronic acid was prepared in porcine plasma (pH = 8.86). 1.5 ml of this solution was placed in the titrating vessel. A total of 60 uL of 0.5 M aqueous solution glucose of same pH were added to the phenylboronic acid solution in 10 uL aliquots. Using a stop watch, the pH was noted after each addition at 10 seconds, 20 seconds, and at equilibrium. Surprisingly, as **FIG. 6** shows, all three plots (10 seconds, 20 seconds, and at equilibrium) of the pH change with glucose concentration were substantially linear over the biologically relevant range. This shows that measurements can be taken quickly and accurately, even when equilibration has not been reached, and without waiting for equilibration. For reproducible results, non-equilibrated measurements should all be taken at the same time when the measurements are to be compared.

EXAMPLE 7

Kinetics of pH Depression in Plasma

10 mL of a 219 mM solution of phenylboronic acid was prepared in plasma and the pH was adjusted to 8.86 using 0.1 N NaOH. A stock solution of 0.5 M glucose was prepared and its pH was also adjusted to 8.86 using 0.01 N NaOH. 10 ul of this glucose solution was added to the plasma solution and every 10 seconds after addition, the pH of the mixture was noted until equilibrium was reached. The same procedure was repeated for the addition of 20 ul, 30 uL, 40 uL, 50 uL, and 60 uL of the stock glucose solution. The resulting concentrations of glucose from these additions are:

10 uL	3.2 mM
20 uL	6.37 mM
30 uL	9.49 mM
40 uL	12.99 mM
50 uL	16.13 mM
60 uL	18.52 mM.

The plot of pH versus time illustrating the kinetics of equilibration is shown by **FIG. 7**.

EXAMPLE 8

Effect of Temperature on Glucose Measurement

Two samples of 1.5 ml of a 50.30 mM solution of phenylboronic acid were placed in the titration vessel at 25 °C and at 43 °C respectively. The pH of both solutions were raised to 8.86 using 0.1 N NaOH. 40 uL of a 0.5 M stock solution of glucose (pH = 8.86) was added to each phenylboronic acid solution and the pH changes over time were noted. As illustrated by **FIG. 8**, equilibrium pH is reached more quickly at the higher temperature. The value of the equilibrium pH also depends on temperature, although this dependence is small for small changes in temperature. Generally, the rate at which equilibrium is achieved can be increased by increasing the temperature.

EXAMPLE 9

Buffer Effect of Boronic Acid Compound

When solid boronic acid is added to the test solution, the aqueous dissociation from the boronic acid itself may also contribute to the decrease in pH which results from the complexation reaction with glucose. That is, boronic acid compounds may release protons (and increase pH) without interaction with a diol-containing target molecule. Because the nature of this effect may be dependent upon several factors including pH, concentration of the boronic acid derivative, and the concentration of glucose, it is preferred that this effect be minimized by adopting the following procedure. Other similar procedures may also be employed.

For consistent and reproducible results when solid boronic acid is used, a solution of boronic acid at a desired concentration is prepared and its pH adjusted to the pKa of the particular boronic acid compound or derivative. This solution is lyophilized to give a solid that is half boronic acid and half the boronic acid salt. The resulting solid, having a 1:1 ratio of the acid and salt form of the boronic acid compound, minimizes the effect of the pH decrease from the independent dissociation of the boronic acid group. This effect is illustrated by the following experiment. Two sets of three 1.5 mL samples of plasma with 5.69 mM, 14.02 mM and 22.36 mM were prepared by adding the appropriate amounts of 0.5 M stock solution of glucose. 12.15 mg of either solid phenylboronic acid or 1:1 mixture of phenylboronic acid and its corresponding salt were added to each set of plasma samples and the resulting pH change was noted. The

difference in pH change from the complex of glucose with phenylboronic acid and a 1:1 mixture of phenylboronic acid and its corresponding sodium salt is shown in **FIG. 9**.

EXAMPLE 10

Use of Color Change to Monitor pH

Three vials each having 1 ml of 50 mM aqueous solution of phenylboronic acid (pH = 8.9) were prepared, and a known amount (10 uL, 30 uL and 50 uL) of a 0.5 M aqueous glucose solution (pH = 8.9) was added to each vial. The vials were shaken for two minutes and then a pH paper (colorpHast pH 6.5 - 10.0, EM-Reagents) was dipped to each vial and the pH was measured from the color of the pH paper. The vial having 10 uL of glucose gave a color which corresponds to the color of pH 8.7 of colorpHast indicator strips pH 6.5-10.0 (EM-Reagents). The vial having 30 uL of glucose gave a color which corresponds to pH = 8.5. The vial having 50 uL of glucose gave a color which corresponds to pH = 8.3. When these tests were done using porcine plasma containing glucose at similar concentrations, similar color changes were observed.

EXAMPLE 11

Use of Imprinted Polymer

Preparation of Imprinted Polymer. A 1:1 complex of methyl α-D-glucopyranoside and 4-vinyl phenylboronic acid was prepared using an adapted version of the procedure for making methyl α-D-glucopyranoside 4,6-phenylboronate described by Ferrier, R.J., *J. Chem. Soc.* 2325-2330 (1961). Imprinted polymer was prepared according to the procedure described by Wulff, G. et al., Makromol. Chem. **178**: 2799-2816 (1977). 0.5 g of complex of methyl α-D-glucopyranoside and 4-vinyl phenylboronic acid, 0.75 g of methylmethacrylate, 1.25 g of ethylene dimethacrylate, and 20 mg of azobis(isobutyronitrile) ("AIBN") in 2.5 ml were filled in a tube, carefully degassed by evacuating, sealed under argon and polymerized for 3 days at 80 °C. Afterwards, the tube was cooled, broken and the polymer was milled and sieved. The polymer was extracted with 1 liter of dry acetonitrile. The template was cleaved by extracting with water/methanol (4:1) for 2 days.

Binding of glucose to imprinted polymer. 100 mg of the imprinted polymer was placed in the titrating vessel at 25 °C. 1.5 ml of water was added and the pH was adjusted to 7.4 using 0.1 N NaOH. A known amount of 0.5 M aqueous glucose solution of the same pH was added and

the pH was noted after each addition. This procedure was continued until a total of 200 uL of the stock glucose solution was added to the imprinted polymer. **FIG. 10** shows the plot of the pH change versus glucose concentration for the imprinted polymer.

EXAMPLE 12

Interaction of Dopamine Target with Phenylboronic Acid

The interaction of dopamine with phenylboronic acid was investigated. The procedure was similar to that o f Example 2, except that the pH of both the initial phenylboronic solution and the stock solution of 0.5 M dopamine were adjusted to 9.38. **FIG. 11** shows the increase in pH due to the interaction of dopamine with phenylboronic acid.

EXAMPLE 13

Glucose Sensor Design

A laminated glucose sensor of the invention is illustrated in this example. The laminate comprises a metal electrode, a sensor compartment (having a sensor composition), and a bipolar membrane. The sensor composition is formulated as a hydrogel, by blending or reacting a boronic acid polymer as a complexing agent and a non-ionic or non-ionogenic matrix polymer. This sensor composition is laminated to an interdigitated gold electrode to form a sensor, which is encapsulated within a thin bipolar membrane to form a laminated composite, as shown for example in **FIG. 12**.

As shown, a preferred laminated sensor has three elements:

(1) a solid, electronically-non-conductive, sheet (*e.g.* glass or plastic) upon which is deposited (*e.g.* by photolithographic means) a metallic pattern of electrodes, preferably gold or platinum, and ultrathin, (*e.g.* 1000 Å) such as pairs of interdigitating electrodes, which are connected to an appropriate conductivity-measuring device;

(2) a thin layer of a sensor composition comprising a copolymer or polymer mixture (*e.g.* a vinyl polymer), one component of which is a boronic acid polymer (*e.g.* vinylphenyl boronic acid), and the other component or components comprising a hydrogel, that is, hydrolytically stable, non-ionic, hydrophilic matrix (*e.g.* vinyl) polymer such as hydroxyethyl methacrylate, vinyl methyl ether, vinyl alcohol, and the like; and

(3) a biopolar ion exchange membrane of high ion exchange capacity (*e.g.* of the type used for biopolar electrodialysis), in intimate contact with the matrix layer and extending over the edges thereof.

The membrane should be sealed to the substrate sheet in a manner which isolates the hydrogel layer from ingress or egress of any diffusible components between the layer and the external environment except by transport across the membrane.

These elements can have any configuration or shape. Suitable configurations include a coplanar organization of layers (one layer on top of another). Another suitable configuration is concentric or annular (one layer around another).

In use, the external face of the bipolar membrane is exposed to a target solution containing glucose. With this configuration, the surface electrodes deposited on the substrate sheet can be used to measure the electrical properties of the hydrogel layer, uninfluenced by the electrical properties of either the bipolar membrane, or the overlying target solution.

In a particularly preferred embodiment, a glucose sensor was fabricated by depositing a thin layer of a sensor composition comprising a binary mixture of poly(2-hydroxyethyl methacrylate) hydrogel and poly(vinyl phenyl boronic acid) on the planar surface of an interdigitated gold electrode. This forms a matrix on the electrode, and the boronate anion is immobilized in the matrix. Glucose interacts with the boronic acid (*i.e* with the immobilized boronate anions) to liberate hydrogen ions (H^+) and water, in a reaction that is believed to form a cyclic ester complex between the boronic acid and the sugar. Because the liberated hydrogen ions or protons are held in the matrix, the ionic conductivity of the matrix increases, generally in a smooth linear or proportional manner, with an increasing ambient glucose concentration. Conductivity can be measured as alternating current (AC) impedance, at high frequencies. Thus, as conductivity increases, resistance decreases, and the AC impedance of the matrix correlates directly with the glucose concentration in a solution contacting the matrix. Impedance is preferably measured at low applied AC (alternating current) potentials (*e.g.* about 0.05 to about 1.0 volts) and at moderately high frequencies (*e.g.* in the audiofrequency spectrum, for example about 1-10 kHz), to minimize electrolysis and electrode polarization effects which might otherwise distort the glucose correlation.

Under these conditions, the measured impedance is simply the resistance of the hydrogel matrix, and is determined by the charge, concentration, and mobility of the ionic species present

in the polymer matrix. Brett and Brett, , *Electrochemistry Principles, Methods, and Applications*, Oxford University Press, 224-230 (1993). In the absence of glucose, and/or of extraneous mobile ionic species that may initially be present in the hydrogel, the matrix conductance will be determined solely by hydrogen and boronate ions generated by dissociation of the largely-unionized boronic acid initially present in the matrix. When glucose is present, formation of the glucose and boronic acid complex liberates hydrogen ions as a function of glucose concentration, which causes a corresponding change in conductance and the measured impedance. Thus, the change in impedance is a unique function of the glucose concentration in the sensor composition matrix.

To improve the reproducibility and accuracy and of the glucose sensor, it is advantageous to minimize or prevent the presence of other ions in the sensor composition matrix. Ideally, the only ions present should be the hydrogen ions produced by the reaction between glucose and boronic acid polymer. Other ions might alter the conductivity of the matrix and have an impact on impedance measurements. This might mask or distort the contribution of liberated hydrogen ions and could influence the correlation between impedance and glucose concentration. Thus, other ions in a test sample of biological fluid should be kept out of the matrix or away from the electrode, while glucose is unimpeded. This can be accomplished, for example, by interposing a barrier between the ambient solution and the sensor composition matrix. One technique is to encapsulate the exposed surfaces of the matrix with a bipolar ion-exchange membrane of high exchange capacity.

The barrier should be substantially impermeable to ionic species present either in the matrix or in the ambient (target) solution, but should be freely permeable to glucose (and possibly other non-ionic species) that are present either in the matrix or in the target solution. That is, the barrier should allow glucose to enter the matrix, should prevent undesirable ions from entering the matrix, and should also prevent ions from leaving the matrix (*i.e.* the protons liberated by reacting glucose with boronic acid groups). A preferred barrier for this purpose is a bipolar electrodialysis (ED) membrane or its functional equivalent. This ion exchange membrane is a bilayer laminate. One layer is a cation exchange layer. It contains immobile anions and mobile cations as counter ions, and functions as a strong-acid cation exchanger of high charge density. The other layer is an anion exchange layer. It contains immobile cations and mobile anions as counter ions, and functions as a strong-base anion exchanger of high charge density. The other

layer is an anion exchange layer. The counter ions in each layer, so-called because their charges are equal to the opposite charges of all of the immobile ions in the layer, are preferably selected from those most commonly present in biological fluids, such as sodium and chloride ions. The counter ions are free to exchange with any ions of the same sign or charge which may be present in the solution contacting each layer.

Since each layer of the biopolar membrane contains counter-ions which are free to exchange with other ions of the same charge that may be present in the contacting solution, it is important that the counter-ions initially present in each layer be incapable of (a) altering the electrical conductance of the contacting hydrogel phase, and/or (b) reacting with (e.g., precipitating) other solutes present in the contacting solution. This can most easily be avoided by selecting, as the initial membrane counter-ions, those ions comprising the electrolyte present in the highest concentrations in the target solution or the hydrogel matrix layer. In the case of biological fluids such as blood, plasma, or blood serum, that electrolyte is sodium chloride. Thus, pre-equilibrating the bipolar membrane in neutral, concentrated aqueous sodium chloride solution, followed by thorough rinsing in distilled water, provides a preferred membrane. Most commercially available bipolar membranes are preconditioned in this manner.

Preferably, the cation exchange layer of the membrane is in contact with the target solution, and the anion exchange layer is in contact with the sensor composition (*e.g.* a hydrogel matrix including a complexing agent). This configuration prevents potentially contaminating ions in the target solution (*e.g.* bicarbonate, phosphate, amino acids) from binding to the membrane. Likewise, hydrogen ions and other mobile cations in the sensor composition will not enter or exchange with other ions in the membrane.

Thus, by interposing a bipolar ion exchange membrane between the matrix layer and the test solution, the glucose sensor, (a) excludes transfer of extraneous ions (including buffer ions such as bicarbonate or phosphate) from the test solution into the matrix, and (b) prevents loss of hydrogen ions generated by glucose/boronic acid complexation (or other ions that might have been initially present) from the matrix. Because of its small effective pore-size, the bipolar membrane is essentially impermeable to relatively large non-ionic and solutes (MW greater than ca. 500 Daltons). This blocks access of the matrix to molecules frequently found in biological fluids such as blood and plasma which could alter or compete with the glucose/boronic acid interaction and thus interfere with the glucose-selectivity of the sensor.

This membrane design provides an ion balance or ionic equilibrium at each solution-membrane layer interface, so that ions do not migrate through the membrane. Any ions having the same charge as the membrane layer are repelled, and do not cross the layer. Thus, negatively charged ions (cations) do not cross the cation exchange layer, and instead are exchanged with cations within the cationic layer, where it comes in contacts with an external ion source. Likewise, positively charged ions (anions) do not cross the anion exchange layer, and instead are exchanged with anions within the anionic layer. In this manner, ions remain in solution or in the membrane. Double-layer membranes of this kind are impermeable to most if not all potentially interfering cations and anions. However, both layers of the membrane are cross-linked hydrogels of relatively high water-content, meaning that any uncharged and water-soluble species with a sufficiently low molecular weight can diffuse through the membrane, and indeed will freely permeate the membrane in either direction. Thus, glucose can cross the membrane and reach the sensor composition. High molecular weight materials, including bioploymers such as proteins and long-chain sugars (polysaccharides) that are usually present in biological fluids such as blood and plasma do not permeate the membrane and do not reach the sensor composition. This is a desirable feature of the invention, because such biopolymers may compete with glucose for boronic acid complexation, or they may foul the sensor by absorbing into it, and their presence may distort glucose measurements.

Ultra-thin membranes are preferred, and are membranes which are as thin as possible whiel remaining free of defects and having adequate mechanical strength for the intended use.

In certain embodiments, more than one complexing agent or sensor composition may be used in concert, or an array or sensors can be employed with different complexing agents. One advantageous use of a plurality of sensors would be improved selectivity, for example, to distinguish between two closely related sugars using the different responses produced by different complexing agents. Algorithms to dereconcile or deconvolute such signals are known or can be empirically derived using known means.

EXAMPLE 14

Glucose Sensor Preparation

Sensor Composition

A sensor composition was provided, comprising a blend of poly(vinyl phenyl boronic acid) as a boronic acid complexing agent and poly(2-hydroxyethyl methacrylate) as hydrogel matrix. Poly(vinyl phenyl boronic acid) is a preferred material for use in a glucose sensor, because it is soluble in common organic solvents, but is insoluble in aqueous solution. Thus, it can be conveniently used in aqueous biological fluids. In addition, the pKa of phenyl boronic as is 8.86, which provides a measurable pH change upon interaction with glucose, within a pH range of between about 6.5 to about 10.

For preparation of the boronic acid polymer, the boronic acid monomer compound 4-vinyl phenyl boronic acid (98%) was obtained from Aldrich Chemical Co., Milwaukee, WI. The hydrogel, for use as a matrix, poly(2-hydroxyethyl methacrylate) with a molecular weight of about 300,000 was also obtained from Aldrich, as were the reagents N-methyl-2-pyrrolidone (NMP, 99%) and AIBN (98%). These materials were used as received.

Poly(vinyl phenyl boronic acid) was prepared by dissolving 0.5 g of vinyl phenyl boronic acid monomer in about 5 ml of methanol with about 5 mg of AIBN. After about 5 minutes of degassing under bubbling argon, polymerization was carried out at about 65 °C for about 12 hours. A pale yellowish solid was obtained. This solid was washed with a small amount of 2X diethyl ether to remove residual solvent, and was subsequently dried in a vacuum at room temperature for about 24 hours.

A 2 wt% (2 g / 100 ml) solution of a 25/75 blend (a 1:3 weight ratio mixture) of poly(vinyl phenyl boronic acid) and poly(2-hydroxyethyl methacrylate) in NMP was prepared. 40 ul of this solution was carefully spread onto the surface of the interdigitated gold electrode. The solvent was then evaporated at about 70 °C for about 60 minutes. The final dry coating thickness was estimated to be ca. 20 micrometers. The coated electrode was then equilibrated with an aqueous solution at a pH of about 7.4 for about 12 hours. After equilibration, the resulting sensor, comprising the sensor layer (boronic acid polymer and hydrogel) and the electrode, was carefully coated with a very thin layer of epoxide glue on four edges. A piece of partially dry bipolar membrane (1x1 cm) was then carefully laid on top of the sensor layer and in contact with the glue-coated edges. After 20 minutes of curing in air, the membrane was firmly secured. The final

assembled sensor was equilibrated with 0.150 M NaCl at a pH of 7.4 before use. The impedance of the sensor in equilibrium with glucose-free 0.150 M NaCl solution was used as the baseline impedance.

Metal Electrode

The electrode was an interdigitated gold electrode obtained from Abtech Scientific Inc., Yardley, PA. This electrode has 50 digit pairs or "fingers" each of which is about 15 um wide and 5 mm long. These fingers are photolithographically deposited on sheets of borosilicate glass. The spacial periodicity is about 60 um. That is, each side of the electrode has fingers that are each 15 um wide, and the distance from the leading edge of one finger to the leading edge of the next finger is 60 um. The fingers on the other side of the electrode have the same periodicity, but are shifted by 30 um, to form a 15 um gap between every pair of fingers. The electrode was cleaned with acetone for about 30 minutes under sonication before use.

Bipolar Membrane

The bipolar membrane was a laminate approximately 250 micrometers thick comprised of a layer of crosslinked sodium polystyrene sulfonate bonded to a layer of crosslinked poly(vinyl benzyl trimethyl ammonium chloride), provided by Aqualytics Company, Warren, NJ. The membrane was originally stabilized with 10% NaCl at neutral pH. Immediately before use, it was equilibrated overnight with 0.150 M NaCl at a pH of about 7.4.

A bipolar membrane will retain its unique impermeability to ionic species while remaining freely permeable to nonionic species, provided that the solutions contacting either layer of the membrane do not contain ionic species which can bind to the fixed charges in the membrane layers and screen the electrostatic charges thereof. In that event, the membrane would lose its co-ion-exclusion properties, and the laminate could become permeable to undesirable cations and/or anions. The majority of biological ionogenic species present in blood and other biological fluids which are capable of these binding interactions tend to carry negative charges in solution at physiological pH (*e.g.* 6.8-7.4). Hence, it is preferable to position the bipolar encapsulating membrane so that the cation exchange layer of the membrane laminate faces outward, that is, toward the target solution. The other layer of the membrane (the anion exchange layer) should prefarbly face inward, that is, toward the sensor composition or layer (the boronic acid polymer and hydrogel matrix). With this orientation, the likelihood of "poisoning" of the membrane with undesirable components from the target solution can be avoided or minimized.

EXAMPLE 15

Use of Laminated Glucose Sensor

Impedance measurements were performed using a CHI-660 instrument (CH Instruments, Cordova, TN) controlled by a Pentium-based personal computer, with insulated leads connecting to the electrode terminals. The sensor was placed in a reaction vessel (a glass cuvette) containing 2.0 ml of 0.150 M NaCl solution (as a control), or in porcine plasma (as a test sample), at a pH of about 7.4. Porcine plasma originally containing about 5 mM glucose was supplied by Becton Dickinson Company, Research Triangle Park, NC and was used as received. Before taking any measurements, the sensor was equilibrated with control solution or test sample for 1 hour, under an applied current of 5 mV and 1 KHz scanning conditions to obtain a stable baseline. Glucose (0.25 M at pH 11.00), obtained from Aldrich was injected stepwise into the reaction vessel under mild stirring. Impedance of the electrode in the sensor was recorded as a function of reaction time.

The measurement being made on this sensor electrode is the AC impedance or conductivity of the sensor layer or gel matrix (boronic acid polymer and hydrogel) lying between the interdigitated gold fingers or conductors upon which the matrix has been deposited. The primary source of this conductivity is the ionic conductance (reciprocal of the impedance) of mobile cations and/or anions present in the matrix.

At the employed AC frequency of 1.0 kHerz (which, with Maxwell Boltzmann kinetics, is virtually equivalent to zero frequency for ion migration), the measured impedance should, for all practical purposes, be the reciprocal of the DC conductivity of the matrix. At the low electrical potentials applied, there should also be no electrochemical processes occurring at the electrodes, and the capacitive contributions to the impedance attributable to electrical double layer polarization and restricted ion mobility should be negligible.

Using this rationale, the measured conductance (reciprocal of the impedance) of the electrode under any given set of operating conditions is directly proportional to the sum of the concentrations of each mobile ion present in the matrix, multiplied by the electrophoretic mobility of the corresponding ion. Consequently, if the electrode matrix (the sensor layer and electrode) is shielded from access to most or all mobile ions present in the target (glucose-containing) solution, *e.g.* by a membrane, but has free access to glucose, and if the only changing mobile ionic species in the matrix is hydrogen ion (produced by the glucose and boronic acid complex), then

the change in conductance of the electrode will be directly and accurately proportional to the change in hydrogen ion concentration in the matrix. This concentration will, in turn, be determined by the glucose concentration in the matrix.

EXAMPLE 16

Glucose Concentration Measurements

The sensor described in Examples 14 and 15 was evaluated under a variety of conditions. **FIGS. 13A** and **13B** for example show the impedance response of the electrode of the sensor as a function of glucose concentration. **FIGS. 13E** and **13F** show the same relationships, plotted in conductance units. In this experiment, the sensor is exposed to a relatively large volume (about 2 ml) of glucose-containing 0.150 M NaCl solution whose glucose concentration is increased stepwise. As the glucose concentration increases from 2.5 to 20 mM, the impedance of the electrode correspondingly decreases to an asymptotic value (over a period of about 30 seconds) with each increment in glucose concentration. The monotonic and nearly linear decrease in the asymptotic impedance (increase in conductance) with increasing glucose concentration is clearly evident. The maximum of the derivative of the impendance also shows a strong linear correlation to glucose concentration, as shown by **FIGS. 13C** and **13D.** Thus, glucose concentration may also be obtained from the derivative of the original signal. Since this maximum occurs much earlier in the impedance-vs-time curve than the time required for the impedance to reach its terminal constant value, monitoring the derivative or slope of the impedance/time curve following exposure of the sensor to a test solution may be a useful strategy for improving the response time of the sensor to changes in test-solution glucose concentration.

The addition of hydrogel significantly enhances the performance of the sensor. When sensors were formed from boronic acid polymer laminated to the electrode, without hydrogel, the sensor layer tended to crack and separate from the electrode surface. In contrast, a 25/75 percent blend of poly(vinyl phenyl boronic acid) and poly(2-hydroxyethyl methacrylate) hydrogel provided a smooth sensor layer that remained intact after several days in solution. In addition to these improvements in adhesion and mechanical properties, the hydrogel is believed to facilitate the diffusion of glucose and subsequent release of protons by increasing the hydrophilicity (proton or hydrogen attraction) or decreasing the hydrophobicity (proton or hydrogen repulsion) of the resulting polymer matrix.

EXAMPLE 17

Membrane Encapsulated Embodiment

The use of a bipolar membrane to encapsulate the sensor layer and electrode significantly improved performance. As shown in **FIG. 14**, an encapsulated sensor (an interdigitated gold electrode coated with poly(vinyl phenyl boronic acid) and poly(2-hydroxyethyl methacrylate)) displayed an approximately five-times higher response to glucose (a) than the same sensor without a bipolar membrane (b). Since there is no pathway for escape of hydrogen ion from the matrix, the hydrogen ion concentration will rise to a level at which the relative concentrations of glucose, glucose/boronic acid complex, and hydrogen ion are in thermodynamic equilibrium. On the other hand, in the absence of membrane encapsulation of the matrix, not only glucose but also other (ionic and non-ionic) species present in the target solution are free to enter the matrix, and hydrogen ions liberated by glucose/boronic acid complexation are free to exchange with other cations and diffuse into the bulk of the target solution. Since blood and most biological fluids contain buffer solutes which prevent significant changes in pH on acid or base addition, the rise in hydrogen ion concentration in the matrix with glucose/boronic acid complexation is substantially depressed. Hence, the signal-amplification benefit provided to this sensor by the the biopolar membrane is a significant feature of the invention.

EXAMPLE 18

Glucose Concentration in Plasma

The performance of the encapsulated sensor in pig (porcine) plasma, a complex biological fluid, is shown in **FIG. 15**. The measured impedance of electrode decreases smoothly as a function of increasing glucose concentration, as demonstrated by adding known amounts of glucose to the plasma, which originally contained about 5 mM glucose. The results are almost identical to those observed with pure glucose solutions, confirming that extraneous components of the plasma have little or no significant influence upon sensor response.

These experiments show that glucose concentration can be simply and accurately determined with a high degree of accuracy and within the clinically relevant range of about 0 to 25 mM.

Upon completion of each series of measurements, the sensor was removed from the test solution, allowed to equilibrate with 0.15 M NaCl solution overnight to remove absorbed glucose,

and the baseline impedance was rechecked. In these experiments the impedance was observed to return to about 50 to 70 % of its initial value. This suggests that the rate of dissociation of glucose from the glucoboronate complex, which requires reprotonation of the boronic acid, becomes slow as the matrix pH approaches its baseline (glucose-free) value. This may influence the accuracy of the sensor at particularly low glucose concentrations in the target solution. One way to eliminate or alleviate this concern would be to slightly acidulate the matrix with a strong acid to depress the baseline pH prior to membrane-encapsulation, provided that the the ionization constant of the glucoboronic acid complex is sufficiently higher than that of the boronic acid compound.

EXAMPLE 19

Sensor Reversibility

The impedance response to decreasing glucose concentrations was also measured, to characterize the reversibility of the sensor. Experiments show that a sensor according to Example 14 responds to decreasing as well as increasing glucose concentrations. For example, the impedance increase observed after decreasing glucose from 10 to 5 mM was $\Delta Z = +7\ \Omega$, and equals the impedance decrease observed when the glucose concentration is increased by the same amount (**FIG. 13B**). In this experiment the response time increased from 30-40 seconds (for the glucose increase) to more than 5 minutes (for the glucose decrease). This may reflect a relatively slow dissociation of glucose from the glucoboronate complex, which requires reprotonation of the boronic acid. The response time may also be affected by the diffusion of glucose across the bipolar membrane. A faster response time may be achieved, for example, by increasing the rate of dissociation of the glucoboronate complex. This could be done for example by selecting a boronic acid complexing agent with a weaker dissociation constant, by addition of a catalyst, or by increasing the temperature. Alternatively, a kinetically-labile copper complex within an alkaline hydrogel matrix may be used for a faster reversible response to fluctuating glucose concentrations.

After completing each series of measurements in this experiment, the sensor was removed from the test solution, allowed to equilibrate with 0.15 M NaCl solution overnight to remove absorbed glucose, and the baseline impedance was rechecked. The impedance did not recover its initial value, an observation known as "drift." This may be due to slow hydrolysis of the hydrogel

(*e.g.* poly(HEMA)) to yield ethylene glycol and methacrylic acid. Another possibility is slow complexation of the hydroxyl functionalities on the hydrogel with boronic acid to yield a complex of somewhat higher acidity than phenyl boronic acid. Another perhaps more likely possibility is an imperfect seal around the bipolar membrane, which may allow very slow leakage of electrolytes from the target solution into the hydrogel, thereby elevating the conductance slightly. This would not be detectable in the presence of glucose at reasonable concentrations, but would raise the baseline conductance of the hydrogel. Ultimately, when immersed in distilled water, *i.e.* for equilibration, the electrolyte that leaked in would slowly leak out -- but over a longer timespan than the duration of the experiment. Nevertheless, despite the evidence of drift, the response of the regenerated sensor to glucosein this experiment was the same as that shown in **FIG. 13B**.

EXAMPLE 20

Glucose/Boronic Acid Complexation Equilibrium

When a glucose sensor according to the invention is first immersed in a test or target solution, glucose present in the solution will diffuse through the bipolar membrane and enter the (initially glucose-free) hydrogel layer. Glucose molecules in the hydrogel are free to interact with the boronic acid groups bound to the gel matrix to form the glucoboronic acid, which then dissociates into the glucoboronate anion and a hydrogen ion. This process will continue (with attendant decrease in pH of the matrix) until the concentration (or more precisely, the chemical activity) of free glucose in the matrix ($[G_M]$, in gmol/ml of water in the matrix) is substantially identical to that in the target solution. If the volume of target solution contacting the matrix is much greater than that of the matrix and membrane, this terminal or steady-state glucose concentration will be essentially the concentration of glucose that was initially present in the target solution ($[G_T]$).

Within the hydrogel matrix, at any instant when the free glucose concentration is $[G_M]$, three equilibrium processes are simultaneously satisfied.

Where B represents a boronic acid group of a boronic acid compound ($R\text{-}B(OH)_2$), and H is a dissociable hydrogen cation of the boronic acid group, the first reaction is the glucose-independent ionization of phenylboronic acid:

(1) $$BH \leftarrow \xrightarrow{K_{A(BH)}} \rightarrow B^- + H^+$$

(2) $$\text{where } K_{A(BH)} = \frac{[B-][H+]}{[BH]}.$$

The second and third reactions are the formation of glucoboronic acid from glucose and boronic acid (*i.e.* the boronic acid compound), and ionization of the resulting glucoboronic acid:

(3) $$BH + G \leftarrow \xrightarrow{K_{(BGH)}} \rightarrow BGH \leftarrow \xrightarrow{K_{A(BGH)}} \rightarrow BG^- + H^+$$

(4) $$\text{where } K_{(BGH)} = \frac{[BGH]}{[BH]\,[G_M]} \text{ and}$$

(5) $$K_{A(BGH)} = \frac{[BG-]\,[H+]}{[BGH]}.$$

There are two other constraints imposed on the isolated hydrogel matrix system. One is the stoichiometric conservation of boronic acid:

(6) $$[B-] + [BG-] + [BGH] + [BH] = [BH_o],$$

where $[BH_o]$ is the total concentration of phenylboronic acid initially present in the matrix (and is constant). The second constraint is the requirement for electroneutrality:

(7) $$[B^-] + [BG^-] = [H^+].$$

The algebra can be materially simplified by observing that, over the physiologic range of glucose concentrations likely to be encountered in diagnostic practice, and at the initial boronic acid concentration level incorporated in the hydrogel matrix ($[BH_o]$ of the order of 0.8 mmol/ml), the concentrations of boronate and glucoboronate ion present in the matrix will be small compared with the total boronic acid present. In this event, the stoichiometric relation reduces to:

(6A) $$[BGH] + [BH] \cong [BH_o]$$

Under these conditions, Equation (4) can be solved to eliminate the glucoboronic acid concentration:

(8) $$K_{(BGH)} = \frac{[BH_0] - [BH]}{[BH]\,[G_M]}, \text{ or}$$

(9) $$BH = \frac{[BH_0]}{1 + K_{BGH}\,[G_M]}$$

By simultaneous solution of Equations 2, 5, 6a, and 9, a relationship can be derived between the equilibrium hydrogen ion concentration $[H^+]$, the free glucose concentration $[G_M]$, and the total concentration of boronic acid in the matrix:

(10) $$[H+]^2 = \frac{K_{A(BH)}\,[BH_0]\left(1 + \frac{K_{A(BGH)}}{K_{A(BH)}} \bullet K_{BGH}\,[G_M]\right)}{(1 + K_{BGH}\,[G_M])}$$

Equation (10) has two asymptotic limits. As $[G_M] \rightarrow 0$, $[H^+] \rightarrow \{K_{A(BH)}\,[BH_o]\}^{1/2}$. That is, as the concentration of free glucose in the matrix approaches zero, the hydrogen ion concentration in the matrix approaches the hydrogen ion concentration due to ionization of phenylboronic acid at its initial concentration in the matrix. At high glucose concentrations, $[H^+] \rightarrow \{K_{A(BGH)}\,[BH_o]\}^{1/2}$. That is, the hydrogen concentration approaches the concentration that would result if all the boronic acid initially present in the matrix had been completely converted to glucoboronic acid.

As noted above, G_M represents the free glucose concentration in the matrix of the sensor (in mass or moles per unit volume of water in the matrix). When the matrix is in equilibrium with

the target solution, the glucose concentration in the target solution (G_T) is the same as the glucose concentration in the sensor composition or matrix (G_M).

Significantly, the ratio of the maximum to the minimum hydrogen ion concentration is equal to the square root of the ratio of the ionization constant of phenylboronic to that of phenylglucoboronic acid:

$$\frac{[H+]_{GM=0}}{[H+]_{GM=\infty}} = \left(\frac{K_{A(BH)}}{K_{A(GBH)}}\right)^{1/2} \quad (10A)$$

Equation (10) can be converted to dimensionless form by defining a reduced hydrogen ion concentration:

$$Y_H=[H+]/[H+]_{GM=0} = [H+]/(K_{A(BH)}\,[BH_o])^{1/2},$$

and a reduced glucose concentration,

$$X_G = K_{BGH}G_M.$$

This simplifies Equation (10) to:

$$Y_H = \left[\frac{1+\frac{K_{A(BGH)}}{K_{A(BH)}} \bullet X_G}{1+X_G}\right]^{1/2} \quad (10B)$$

Equation (10B) is plotted in **FIG. 16** to show Y_H (reduced hydrogen concentration) as a function of X_G (reduced glucose concentration) for various values of the ratio $[K_{A(BGH)}/K_{A(BH)}]$, and illustrates the dependence of the sensor response upon the ratio of the ionization constants of the phenylglucoboronic acid and phenylboronic acid. For phenylboronic acid, the ionization constant is reported to be $pK_{A(BH)} = 8.84$. In the absence of glucose, $[H+]^2 = (0.8)(1.45 \times 10^{-9})$, or $[H+] =$

3.4×10^{-5} (pH = 4.47). If $K_{A(BGH)} \cong 1.0 \times 10^{-6}$, then the upper asymptotic hydrogen ion concentration is $[H+] = 8.9 \times 10^{-4}$ (pH = 3.05). This means that the pH change of the matrix over a physiologic range of glucose concentrations in the target solution will be of the order of one unit (i.e., ten-fold), if the initial boronic acid present in the matrix is in the form of free boronic acid.

EXAMPLE 21

Titration With Strong Base

The phenylboronic-acid-containing hydrogel matrix can be titrated with a strong base (e.g., sodium hydroxide) to raise the pH to any desired value. (At pH 7.0, for example, less than 2 % of the phenylboronic acid present in the matrix will be converted to sodium phenylboronate.) This changes the accessible range of hydrogen ion concentration variation with glucose concentration from one pH unit to about 4 units, thereby raising the sensitivity of the sensor by a factor of one thousand.

In the absence of glucose, the matrix hydrogen ion concentration $[H_o^+]$ corresponding to any specified sodium ion concentration of $[Na_o^+]$ (where the total boronic acid concentration is $[BH_o]$, and $[Na_o^+] >> [H_o+]$) is determined by the Equation:

$$[H_0^{'+}] \cong K_{A(BH)}\left\{\frac{[BH_0]}{[Na_o^+]} - 1\right\} \qquad (11)$$

At pH 7.0 in the matrix (containing 0.8 gmol/l phenylboronic acid), the concentration of sodium ion will be about 1.2×10^{-2} M, and will not vary with changes in pH that accompany glucose uptake. Thus, if impedance (or conductance) measurements are used to monitor changes in hydrogen ion concentration in the matrix with glucose uptake, the matrix should preferably be partially neutralized with strong base before being applied to the conductive substrate and encapsulated by the bipolar membrane. The optimal degree of neutralization will depend upon the precise range of glucose concentrations over which the sensor is to have maximum sensitivity.

The partial titration of the phenylboronic acid hydrogel matrix with a strong base (e.g., NaOH) will deprotonate part of the acid, but not otherwise effect the equilibrium relationships

developed above. If the added NaOH results in a sodium ion concentration of $[Na_o^+]$ in the matrix, Equation (7) above becomes:

$$[B^-] + [BG^-] = [H'^+] + [Na_o^+]. \quad (7A)$$

Inasmuch as $[Na_o^+]$ is constant and independent of either hydrogen ion or glucose concentration, solving the same equilibrium and mass conservation equations with this change in the neutrality relationship will yield the desired dependence of hydrogen ion concentration in the matrix upon the glucose concentration (and sodium ion concentration). The final relationship (again, with the constraint that $[Na_o^+] << [BH_o]$) is readily shown to be:

$$[H'+]^2 + [H'+][Na_0^+] = \frac{K_{A(BH)}[BH_0]\left(1 + \frac{K_{A(BGH)}}{K_{A(BH)}} \bullet K_{BGH}[G_M]\right)}{1 + K_{BGH}[G_M]} \quad (12)$$

The right-hand sides of Equations (10) and (12) are identical. This relationship, as does Equation (9), predicts a rising hydrogen ion concentration with increasing glucose concentration, with two asymptotic limits. At zero glucose concentration, Equation (12) reduces to:

$$\frac{[H'+]_{G_M=0}}{[Na_0^+]} = \frac{\left\{1 + 4K_{A(BH)} \bullet \frac{[BH_0]}{[Na_0^+]}\right\}^{1/2} - 1}{2} \quad (13)$$

At high glucose concentrations, the equation is:

$$\frac{[H'+]_{G_M=\infty}}{[Na_0^+]} = \frac{\left\{1 + 4K_{A(BGH)} \bullet \frac{[BH_0]}{[Na_0^+]}\right\}^{1/2} - 1}{2} \quad (14)$$

In this case, the ratio of the minimum to maximum hydrogen ion concentrations is dependent not only on the ratio of the ionization constants of phenylboronic and phenylglucoboronic acids, but also on the fraction of the boronic acid which has been converted to its sodium salt. If this fraction is small (i.e., the ratio of total boronic acid to sodium ion in the matrix is large) a considerably larger decrease in pH with increasing glucose concentration (relative to that obtained with an unneutralized phenylboronic acid hydrogel matrix) will result.

Equation (12) can be solved explicitly for the hydrogen ion concentration as a function of the glucose concentration in the matrix, with the initial boronic acid content, $[BH_o]$, and the sodium ion content, $[Na_o^+]$ as adjustable parameters:

$$(15)\quad \frac{[H'+]}{[Na_0^+]} = \frac{\left\{1+\frac{4K_{A(BH)}[BH_0]\left(1+\frac{K_{A(BGH)}}{K_{A(BH)}}\bullet K_{BGH}[G_M]\right)}{[Na_0^+]^2\,(1+K_{BGH}[G_M])}\right\}^{1/2}-1}{2}$$

Like Equation (10), Equation (15) can be rendered dimensionless by defining a reduced hydrogen ion concentration, $(Y'_H = [H'^+] / [H'^+]_{GM=0})$, and a reduced glucose concentration as in Equation (10b), $(X_G = K_{BGH}G_M)$, leading to an equation of the same general form as that of Equation (10b), but with $[BH_o]$ and $[Na_o^+]$ adjustable parameters:

$$(16)\quad Y'_H = \frac{\left\{1+\frac{4K_{A(BH)}[BH_0]\left(1+\frac{K_{A(BGH)}}{K_{A(BH)}}X_G\right)}{[Na_0^+]^2(1+X_G)}\right\}^{1/2}-1}{\left(1+\frac{4K_{A(BH)}[BH_0]}{[Na_0^+]^2}\right)^{1/2}-1}$$

This relationship has been plotted in **FIG. 17** as a function of the ratio $[BH_o]/[Na_o+]$ (for a constant ratio of $K_{A(BGH)}/K_{A(BH)} = 690$). As expected, partial neutralization of the boronic acid increases the hydrogen ion concentration in the matrix (relative to its value in the absence of glucose) at any glucose concentration, the increase becoming more marked at higher extent of neutralization and higher glucose levels. Since, however, the absolute value of the hydrogen ion concentration in the glucose-free matrix decreases rapidly as the fraction of boronic acid neutralized increases, the absolute value of the hydrogen ion concentration at any glucose concentration may also decrease. For this reason, enhanced conductimetric sensitivity of the sensor is likely to be realized at relatively low levels of matrix neutralization, where the hydrogen ion concentration is a significant fraction of the total cation concentration (i.e., $[H^+] + [Na_o^+]$).

Possibly, a more helpful indication of the effect of partial neutralization of the phenylboronic acid upon the hydrogen ion concentration in the matrix at any specified glucose concentration can be achieved by comparing that value with the hydrogen ion concentration (at the same glucose concentration) without such neutralization. This can be approximated by dividing Equation (15) by the square root of Equation (10), yielding:

(17)

$$\frac{[H'+]}{[H+]} = R = \frac{\frac{[Na_0^+]}{2}\left\{\left(1+\frac{4K_{A(BH)}[BH_0]\left(1+\frac{K_{A(BGH)}}{K_{A(BH)}}X_G\right)}{[Na_0^+]^2(1+X_G)}\right)^{1/2}-1\right\}}{\left(\frac{K_{A(BH)}[BH_0]\left(1+\frac{K_{A(BGH)}}{K_{A(BH)}}X_G\right)}{1+X_G}\right)^{1/2}}$$

where R is defined as the "relative hydrogen ion concentration", i.e., the ratio of the hydrogen ion concentration in the partially-neutralized matrix to that in an unneutralized matrix (both at the same value of X_G). The two adjustable parameters affecting this ratio are (1) the ratio of total boronic acid to sodium ion in the matrix ($[BH_o]/[Na_o^+]$), and (2) the ratio of the

ionization constants of the boronic and glucoboronic acids ($K_{A(BGH)}/K_{A(BH)}$). Plots of (R) vs. (X_G) as with the above two ratios as parameters are shown in **FIGS. 18** and **19**.

In **FIG. 18**, the quantity R has been plotted as a function of the ratio $[BH_o]/[Na^+_o]$ for various values of (X_G), using the values $K_{A(BH)} = 1.45 \times 10^{-9}$ mol/l, $[BH_o] = 0.8$ mol/l, and $K_{A(BGH)}/K_{A(BH)} = 690$. This plot indicates that the extent of pre-neutralization of the boronic acid should be kept to very low levels for maximum enhancement in hydrogen-ion sensitivity, and that the benefits of pre-neutralization become important even at quite low glucose concentrations in the test solution.

In **FIG. 19**, R has been plotted vs. X_G for several values of the ionization constant ratio ($K_{A(BGH)}/K_{A(BH)}$) for a fixed fraction of matrix neutralization ($[BH_o]/[Na_o^+] = 1000$). R increases monotonically with ($K_{A(BGH)}/K_{A(BH)}$) at all glucose concentrations, indicating that the stronger the glucoboronic acid is, relative to the boronic acid, the less the benefit to be gained from (or the less the need for) partial neutralization of the matrix as a means of enhancing conductimetric sensitivity.

EXAMPLE 22

Variation of Matrix Conductance With Glucose Concentration

An aqueous solution of ionized solutes, when subjected to an externally impressed or applied electrical potential, will conduct an electric current at a rate determined by the mobility, charge, and concentration of all ions present in that solution, and the magnitude of the potential gradient to which these ions are subjected. In order for the impressed potential to be directly related to the potential gradient in solution, it is important that charge-transport processes not related to ion migration (e.g., electrolysis, or electrical polarization processes at the electrode/solution interfaces) be avoided or minimized. In conventional conductimetric measurements, this is commonly accomplished by employing low impressed potentials, and a relatively high frequency (ca. 1 kilohertz).

Under these conditions, the conductivity of an electrolyte solution is the sum of the conductivity contributions of the individual ions present in that solution. If the solution is dilute, the conductivity contribution of each ion is determined solely by the ion mobility in solution and its coulombic charge or valence; this quantity is the molar equivalent conductivity (λ), in units of mho (or Siemens) x cm^2/ gmol. For a solution of electrolytes, the equivalent ionic conductivity of that solution (Λ) (defined as the measured conductivity divided by the molar concentrations of all ions present) is given by:

(18) $$\Lambda = \Sigma\lambda_- + \Sigma\lambda_+$$

For a solution containing a mixture of electrolytes whose ions are all of the same valency, the specific conductivity the solution, (κ) (mho/cm) is given by:

(19) $$\kappa = \Sigma\lambda_- c_- + \Sigma\lambda_+ c_+, \text{ where } \Sigma c_- = \Sigma c_+.$$

A hydrogel of relatively high water content (e.g., 50 % water by volume or greater) can be expected to have electrical conductivity characteristics very similar to those of an aqueous solution containing the same electrolytes at the same molar concentrations. Since conductance is determined by concentration of charge-carriers on a total volume basis, the molar concentration of each ion should be computed on the basis of mols/ ml of total gel volume. In contrast, the

thermodynamic activity an ion in a hydrogel is usually best estimated in terms of the ion concentration in mols/ml of water in the gel. This is usually because the ions are almost completely excluded from the polymer phase.

In the hydrogel matrix phase of the sensor, the ionic species likely to be found in significant concentration include hydrogen ion (H^+), phenylboronate ion ($R\text{-}B^-$), phenylglucoboronate ion ($R\text{-}BG^-$), and sodium ion (Na+) if partial neutralization of the gel has been carried out with NaOH. For such a gel the specific conductivity will be given by the relation:

$$\kappa = \lambda_{Na+}C_{Na+} + \lambda_{H+}C_{H+} + \lambda_{B-}C_{B-} + \lambda_{BG-}C_{BG-}. \quad (20)$$

However, the phenylboronate ions and phenylglucoboronate ions are covalently coupled to the gel matrix, where their mobility in an electric field is virtually nil. Under these conditions the charge carriers in the gel are almost exclusively the sodium and hydrogen cations. This simplifies the specific conductivity as follows:

$$\kappa \cong \lambda_{Na+}C_{Na_o} + \lambda_{H+}C_{H+} . \quad (21)$$

The concentration of sodium ion in the matrix, C_{Nao}, is constant and independent of either glucose or hydrogen ion concentration. However, in the absence of glucose, the hydrogen ion concentration in the matrix is determined solely by the ionization constant of phenylboronic acid as required by Equation (2) above.

At room temperature, the equivalent molar conductivities of the sodium and hydrogen ions are reported to be 350 and 126 mho cm^2/g mol, respectively. The unusually high mobility of hydrogen ion makes its accurate determination by conductivity measurement even in the presence of other ions relatively straightforward.

The macroscopic conductance of the hydrogel compartment of the sensor ($\mathcal{G}$ in mhos), i.e. the reciprocal of its measured electrical impedance in ohms, is given by:

$$(22) \qquad e = \alpha [\lambda_{Na+} C_{Na_o} + \lambda_{H+} C_{H+}]$$

where (α) is a geometric constant of the hydrogel compartment (determined by electrode area and mean separation distance between electrodes), and all other parameters, as previously defined.

In the absence of glucose, the matrix conductivity is given by Equation (19), where $e =$ e_α, and C_{H+} is replaced with C_{Ho+} from Equation (2A):

$$(22A) \qquad e = \alpha [\lambda_{Na+} C_{Na_o} + \lambda_{H+} C_{Ho}]$$

whereupon subtraction of Equation (22A) from Eq. (22) yields:

$$(23) \qquad e - e_\alpha = \alpha \lambda_{H+} (C_{H+} - C_{Ho+})$$

Equation (23) relates the increase in matrix conductivity directly to the increase in hydrogen ion concentration resulting from the presence of glucose at any specific concentration in the matrix. All that is required to determine the glucose concentration in the target solution (at equilibrium) is the difference between the "reference" conductivity of the matrix in the absence of glucose, and that of the matrix in the presence of glucose.

By inserting into Equation 23 the value for C_{H+} from Equation (12), and the value of C_{Ho+} from Equation (2A), the change in conductivity of the matrix compartment (over the "reference" value in the absence of glucose) can be directly related to the glucose concentration, and thus correlate the matrix conductivity with the glucose concentration in the matrix and (at equilibrium) to the glucose concentration in the target solution. The final relationship is:

$$(24)\quad e\text{-}\ e_u = \alpha\lambda_H + K_{A(BH)}\left(\frac{[BH_0]}{[Na_o^+]} - 1\right)\left\{\frac{(1 + K_{BGH})\dfrac{K_{A(BGH)}}{K_{A(BH)}}[G_M]}{1 + K_{BGH}[G_M]} - 1\right\}.$$

At low glucose concentrations, the changes in conductivity are nearly linear in glucose concentration. At high glucose concentrations, the conductivity becomes nearly constant and independent of glucose concentration. Also, as noted earlier, the larger the fraction of phenylboronic acid in the matrix that has been deprotonated with NaOH, the smaller will be the changes in matrix conductivity with changing glucose concentration.

EXAMPLE 23

Dynamics of Sensor Conductivity Response to Variations in Glucose Concentration

The preceding analysis allows prediction of the glucose concentration in a target solution under conditions where the sensor has been allowed to reach equilibriium between the hydrogel matrix compartment and the target solution with respect to glucose. Under these conditions, the free glucose concentration in the matrix is equal to that in the target solution, and the hydrogen ion concentration in the matrix is determined by the equilibrium concentrations of all boronic acid species present, as shown in Equations (12) and (21).

The time required for this equilibrium condition to be reached from the instant the sensor surface is contacted with the target solution is dependent upon (1) the rate of transport of glucose across the bipolar membrane, (2) the rate of diffusion of glucose from the matrix-side of the membrane into the hydrogel, and (3) the kinetics of interaction of the glucose with the boronic acid and the rate of hydrogen ion liberation from the glucoboronic acid complex.

In many instances, the most probable rate-limiting process in this sequence will be the transport of glucose across the bipolar membrane. If the membrane is sufficiently thin that the amount of glucose impounded within the membrane (that is, the average solubility of glucose in the membrane when half-saturated with glucose from the target solution) is but a small fraction of the total glucose incorporated in the matrix at equilibirum, then the rate of transport of glucose across the membrane into the matrix ($dn_G/dè$, in gmol/sec) be approximated by the "pseudo-steady-state" membrane transport relationship:

(25) $dn_G/d\theta = P_G A_m/t_m ([G_T] - [G_m])$

where (P_G) is the permeability coefficient of glucose in the membrane, (A_m) and (t_m) are the area and thickness of the membrane, respectively, and $[G_T]$ and $[G_m]$ are the glucose concentrations in the target solution and hydrogel matrix at any time, respectively.

If, on the other hand, the membrane is relatively thick, and/or the glucose permeability is low, the rate of delivery of glucose into the hydrogel layer will be governed by unsteady-state diffusion of glucose into the membrane until a linear concentration gradient is established, following which Equation (25) will apply. This will result in a gradual increase in the rate of change of hydrogel conductance with time until the "pseudo steady state" is reached. The "lag-time" (θ) required to achieve the "pseudo steady state" condition is approximated by the relation: è = $t^2/6D$, where (t) is the membrane thickness, and (D), the diffusivity of glucose in the membrane. Reasonable values for the membrane thickness and glucose diffusivity are, respectively, 50 micrometers and 2 x 10^{-6} cm^2/sec. For this system, the lag-time will be only about two seconds. Hence, a sensor of this type and configuration provides a quite rapid response to step-changes in glucose concentration in the test solution.

Material balance and electroneutrality constraints in the matrix require that:

(26) $n_G = v_m ([G_m] + [BGH] + [BG^-])$;

(27) $[BH] + [BGH] + [B^-] + [BG^-] = [BH_o]$; and

(28) $[B^-] + [BG-] = [Na_o^+] + [H^+] \cong [Na_o^+]$

where (v_m) is the volume of hydrogel in the matrix compartment.

These equations, in combination with the equilbrium relations for the formation of glucoboronic acid and ionization of boronic and glucoboronic acid, can be simultaneously solved and reduced to:

(29) $$n_G = v_m[G_m]\left(1+\frac{K_{BGH}[BH_0]}{1+K_{BGH}[G_m]}\right)$$

Equation (24) relates the change in matrix conductivity when the glucose concentration is any value $[G_m]$ to that in the absence of glucose, and thus can be used to determine the change of conductivity corresponding to any change in glucose concentration in the matrix.

Differentiation of Equaitons (24) and (29) with respect to time, and application of the transport rate equation, Eq. (25), will allow determination of the changes in matrix conductivity with time following exposure of the sensor to any specified glucose concentration in the target solution.

It is clear from this analysis that, if the matrix conductivity and its changes with variations in glucose (or hydrogen ion) concentration can be measured with high accuracy, then from Equation (25), if the membrane is sufficiently thin and glucose-permeable, the glucose concentration in the target solution can be determined in a very short time from the initial slope of the conductivity/time curve. This allows very rapid measurement of glucose levels, and permit real-time monitoring of glucose concentrations in blood and other body fluids with indwelling sensors.

The invention is applicable to the design and fabrication of a variety of chemical analytical sensors where (a) the solute to be monitored is non-ionic, (b) a non-ionic or weakly ionized solute-specific complexing agent is available, and (c) the complexation process liberates one or more ions. Commercially available microminiaturization technology can be utilized to provide fabrication of devices of very small size, high reproducibility, and low cost, suitable for use in environmental monitoring and industrial measurement and control systems as well as for laboratory and *in vivo* biomedical applications. Furthermore, since monitoring of the sensor element involves the use of a simple low-voltage audiofrequency generator and a microammeter, the instrumentation package supporting this device can be of similar high stability, compactness, and low cost. It will be understood by practitioners of ordinary skill that the invention can be made, used and applied in a variety of ways, and is not limited by the particular examples set forth herein. Moreover, it will be understood that the invention and claims are not limited by any theory or theories set forth herein.

What is claimed is:

1. A sensor for analysis of a target molecule in solution, comprising a gel having a matrix polymer and a complexing agent, wherein interaction of the complexing agent with the target molecule produces detectable ions.

2. A sensor of claim 1, wherein the target molecule is a non-ionic solute in an aqueous solution, and the gel is a water-insoluble hydrogel.

3. A sensor of claim 2, wherein the complexing agent is a boronic acid compound.

4. A sensor of claim 3, wherein the boronic acid compound is a polymer selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

5. A sensor of claim 2, wherein the pKa of the complexing agent is more than about 7.8.

6. A sensor of claim 4, wherein the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether).

7. A sensor of claim 1, additionally comprising at least one electrode in contact with the hydrogel.

8. A sensor of claim 2, additionally comprising at least one electrode in contact with the hydrogel.

9. A sensor of claim 8 wherein the electrode is an interdigitated metal electrode made using at least one of copper, silver, gold and platinum.

10. A sensor of claim 8 wherein a low voltage alternating current potential in the audiofrequency spectrum is applied to the electrode.

11. A sensor of claim 9 wherein a low voltage alternating current potential in the audiofrequency spectrum is applied to the electrode.

12. A sensor of claim 8, wherein

the boronic acid compound is a polymer selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid);

the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether) and

the electrode is an interdigitated metal electrode.

13. A sensor of claim 2 additionally comprising at least one of a stabilizer and a preservative.

14. A sensor of claim 12 additionally comprising at least one of a stabilizer and a preservative.

15. A sensor of claim 2 wherein the ratio of complexing agent to matrix polymer is from about 1:3 to about 3:1.

16. A sensor of claim 4 wherein the ratio of complexing agent to matrix polymer is from about 1:3 to about 3:1.

17. A sensor of claim 12 wherein the ratio of complexing agent to matrix polymer is from about 1:3 to about 3:1.

18. A sensor of claim 2 wherein the target molecule is a diol compound.

19. A sensor of claim 3 wherein the target molecule is a cis-diol compound.

20. A sensor of claim 4 wherein the target molecule is a sugar compound.

21. A sensor of claim 8 wherein the target molecule is glucose.

22. A sensor of claim 12 wherein the target molecule is glucose.

23. A sensor of claim 22 wherein the electrode is an interdigitated gold electrode.

24. A sensor of claim 1 additionally comprising a semipermeable barrier in contact with the gel and interposed between the gel and the solution.

25. A sensor of claim 2 additionally comprising a semipermeable barrier in contact with the hydrogel.

26. A sensor of claim 25, wherein the semipermeable barrier surrounds the hydrogel.

27. A sensor of claim 25, wherein the semipermeable barrier is laminated to the hydrogel.

28. A sensor of claim 25, wherein the semipermeable barrier is a bipolar ion-exchange membrane.

29. A sensor of claim 12, wherein the hydrogel is in contact with a bipolar ion-exchange membrane.

30. A sensor of claim 12, wherein the electrode is laminated to the hydrogel.

31. A sensor of claim 2 wherein the produced ions are detected by evaluating at least one of a change in hydrogen ion concentration, acidity, pH, conductance, and impedance.

32. A sensor of claim 8 wherein the produced ions are detected by evaluating at least one of a change in hydrogen ion concentration, acidity, pH, conductance, and impedance.

33. A sensor of claim 12 wherein the produced ions are detected by evaluating at least one of a change in hydrogen ion concentration, acidity, pH, conductance, and impedance.

34. A sensor of claim 29 wherein the produced ions are detected by evaluating at least one of a change in hydrogen ion concentration, acidity, pH, conductance, and impedance.

35. A sensor of claim 2 wherein one or more components of the hydrogel is a polymer that is imprinted with one of a target molecule and an analog of a target molecule.

36. A sensor of claim 2 wherein the aqueous solution is a biological fluid.

37. A sensor of claim 29, wherein the hydrogel is laminated to the electrode and the bipolar ion-exchange membrane is laminated to the hydrogel.

38. A sensor of claim 29 wherein the bipolar ion-exchange membrane surrounds the electrode and hydrogel.

39. A sensor of claim 38 wherein the bipolar ion-exchange membrane is ultra-thin.

40. A sensor of claim 37, wherein the bipolar ion-exchange membrane has a cationic layer and an anionic layer, and the anionic layer is in contact with the hydrogel.

41. A sensor of claim 24, wherein the complexing agent is a metal complexing agent.

42. A sensor of claim 41, wherein the metal complexing agent is a copper (II) complex.

43. A sensor of claim 29, wherein the complexing agent is a metal complexing agent.

44. A sensor of claim 43, wherein the metal complexing agent is selected from the group consisting of copper(II)-triazacyclononane; copper(II)-1,4-dimethyl-triazacyclononane; copper(II)-ethylenediamine; copper(II)-propylenediamine; copper(II)-iminodiacetate; copper(II)-diethylenetriamine; copper(II)-1-oxa-4,7-diazacyclononane; and copper(II)-1-thia-4,7-diazacyclononane.

45. A sensor according to claim 2, wherein the ion-producing interaction between the complexing agent and the target molecule is reversible.

46. A sensor according to claim 45, wherein the concentration of produced ions is a function of the concentration of target molecule.

47. A sensor according to claim 25, wherein the target molecule is non-ionic.

48. A sensor according to claim 29, wherein the concentration of produced ions is a function of the concentration of target molecule.

49. A sensor according to claim 46, wherein the concentration of produced ions is evaluated by at least one of conductimetry, fluorimetry, color change, polarimetry, light scattering, nephelometry, determining pH, assessing acidity, measuring impedance, and specific ion potentiometry.

50. A sensor according to claim 46 that is implantable in a living mammal.

51. A sensor for evaluating the concentration of a non-ionic target molecule in an aqueous test solution having a plurality of ionic and non-ionic ingredients, comprising

a non-ionic complexing agent and a semipermeable membrane,

wherein the membrane permits contact between target molecules in the test solution and the complexing agent, and hinders contact between ionic ingredients in the test solution and the complexing agent; and

wherein the complexing agent reversibly interacts with the target molecule to produce ions in a measurable concentration that indicates the concentration of the target molecule in the test solution.

52. A sensor of claim 51, wherein the complexing agent is combined with a non-ionic and water-insoluble matrix polymer to form a hydrogel.

53. A sensor of claim 51, additionally comprising at least one electrode.

54. A sensor of claim 52, additionally comprising at least one electrode.

55. A sensor of claim 51, wherein the concentration of ions is measured by at least one of conductimetry, fluorimetry, color change, polarimetry, light scattering, nephelometry, determining pH, assessing acidity, measuring impedance, and specific ion potentiometry.

56. A sensor of claim 54, wherein the concentration of ions is measured by at least one of measuring conductance, impedance, and pH.

57. A sensor of claim 52, wherein the complexing agent is a boronic acid compound.

58. A sensor of claim 54, wherein the complexing agent is a boronic acid polymer.

59. A sensor of claim 58, wherein the boronic acid polymer is selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

60. A sensor of claim 52, wherein the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether).

61. A sensor of claim 59, wherein the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether).

62. A sensor of claim 54, wherein the complexing agent is a boronic acid polymer selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

62. A sensor of claim 61, wherein the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether) and poly(vinyl 3,5-dichlorophenylboronic acid).

63. A sensor of claim 51 wherein the semipermeable membrane is a bipolar ion-exchange membrane.

64. A sensor of claim 54 wherein the semipermeable membrane is a bipolar ion-exchange membrane.

65. A sensor of claim 52 wherein the ratio of complexing agent to matrix polymer is about from 1:3 to about 3:1.

66. A sensor of claim 54 wherein the electrode is an interdigitated metal electrode.

67. A sensor of claim 62 wherein the electrode is an interdigitated metal electrode.

68. A sensor of claim 52 wherein the test solution is a biological fluid.

69. A sensor of claim 52 wherein the target molecule is a diol compound.

70. A sensor of claim 58, wherein the target molecule is glucose.

71. A molecular sensor comprising

at least one electrode,

a sensor compartment comprising a non-ionic complexing agent, and

a semipermeable barrier,

wherein the electrode is in communication with the sensor compartment, and

the barrier separates the electrode and sensor compartment from a test solution.

72. A sensor of claim 71, wherein the sensor compartment additionally comprises a hydrogel.

73. A sensor of claim 72, wherein the hydrogel comprises a matrix polymer selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether) and poly(vinyl 3,5-dichlorophenylboronic acid).

74. A sensor of claim 72, wherein the electrode is an interdigitated metal electrode.

75. A sensor of claim 71, wherein the complexing agent is a metal complexing agent.

76. A sensor of claim 72, wherein the complexing agent is a boronic acid compound.

77. A sensor of claim 72, wherein the complexing agent is a boronic acid polymer.

78. A sensor of claim 72, wherein

the complexing agent is selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid); and

the hydrogel comprises a matrix polymer selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether) and poly(vinyl 3,5-dichlorophenylboronic acid).

79. A sensor of claim 78, wherein the ratio of complexing agent to matrix polymer is in the range of about 1:3 to 3:1.

80. A sensor of claim 78, wherein the electrode is an interdigitated metal electrode.

81. A sensor of claim 71, wherein the barrier is a bipolar ion-exchange membrane.

82. A sensor of claim 76, wherein the barrier is a bipolar ion-exchange membrane.

83. A sensor of claim 78, wherein the barrier is a bipolar ion-exchange membrane.

84. A molecular sensor comprising

at least one electrode,

a bipolar ion-exchange membrane, and

a compartment comprising a non-ionic and water-insoluble hydrogel incorporating a non-ionic complexing agent,

wherein the electrode communicates with the compartment, and

the membrane separates the electrode and compartment from a test solution.

85. A sensor of claim 84, wherein the electrode is an interdigitated metal electrode.

86. A sensor of claim 84, wherein the complexing agent is a boronic acid polymer.

87. A sensor of claim 84, wherein the electrode responds to a change in ion concentration in the compartment that is produced by contacting the sensor with a test solution.

88. A sensor of claim 87, wherein the change in ion concentration in the compartment indicates the concentration of a target molecule in the test solution.

89. A sensor of claim 88, wherein the change in ion concentration in the compartment is produced by contacting the complexing agent with a target molecule.

90. A sensor of claim 84, wherein

the electrode is an interdigitated metal electrode,

the complexing agent is selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid); and

the hydrogel comprises a matrix polymer selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether) and poly(vinyl 3,5-dichlorophenylboronic acid).

91. A sensor of claim 84, wherein the electrode forms a first layer, the compartment forms a second layer, and the membrane forms a third layer.

92. A sensor of claim 91, wherein the layers are one of planar and concentric.

93. A sensor of claim 84, wherein the compartment is sandwiched between an electrode layer and a membrane layer.

94. A sensor of claim 84 that is microfabricated, and wherein the membrane is an ultra-thin layer surrounding the compartment and electrode.

95. A sensor of claim 94 that is implantable in a live mammal for continuous monitoring.

96. A sensor of claim 84, wherein the electrode is an interdigitated metal electrode on a solid support, the hydrogel comprises a mixture of poly(2-hydroxyethyl methacrylate) and poly(vinyl phenyl boronic acid) that is immobilized on the solid support and is in contact with the electrode, and the membrane covers and is in contact with the hydrogel.

97. A sensor of claim 96,wherein the membrane comprises a layer of crosslinked sodium polystyrene sulfonate bonded to a layer crosslinked poly(vinyl benzyl trimethyl ammoninum chloride).

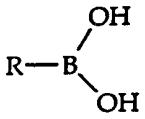
98. A sensor of claim 84, wherein the membrane comprises a cation exchange layer bonded to an anion exchange layer, the anion exchange layer faces the hydrogel, and the cation exchange layer is accessible for exposure to a test solution.

99. A sensor of claim 84 wherein the pKa of the complexing agent is more than about 7.8.

100. A method for determining a concentration of a diol compound in a liquid sample, comprising:

determining a first pH of the sample;

contacting the sample with a known amount of a boronic acid compound of the formula



wherein R is selected from a group consisting of hydrogen, unsubstituted alkyl, substituted alkyl, unsubstituted aryl, and substituted aryl;

determining a second pH of the sample; and,

using the first pH and the second pH to determine the cis-diol concentration of the sample.

101. A method of claim 100 wherein the diol is selected from the group consisting of glycerol, dopamine, catechol, ascorbic acid, ethleneglycol, 3-hydroxy-L-tyrosine and 1,4, anhydroerythritol.

102. A method of claim 100 wherein the diol is a saccharide.

103. A method of claim 102 wherein the saccharide is glucose and the sample is plasma or blood.

104. A method of claim 100 wherein R is phenyl.

105. A method of claim 100 wherein R is a substituted phenyl and the phenyl substitution includes one or more groups selected from the group consisting of C_1-C_5 alkyl, C_1-C_5 alkoxy, hydroxyl, thiol, thioether, ketone, aldehyde, epoxy, ester, ether, amine, imine, amide, nitro, carboxylic acid, disulfide, carbonate, isocyanate, carbodiimide, carboalkoxy, carbamate, and halogen.

106. A method of claim 100 wherein the boronic acid compound is selected from the group consisting of phenylboronic acid, *p*-methoxyphenylboronic acid, *p*-methylphenylboronic acid, and *m*-nitrophenylboronic acid.

107. A method of claim 100 further comprising heating the sample before determining the second pH of the sample.

108. A method of claim 107 wherein the sample is heated to a temperature between about 40° C and about 45°C.

109. A method of claim 100 wherein the diol concentration is determined within a range of about 0 to 25 mM and with an accuracy of within at least about 1 mM.

110. A method of claim 100 wherein the boronic acid compound is supported by a hydrogel matrix.

111. A method for measuring the concentration of a target molecule in solution, comprising the steps of:

exposing a target molecule to a gel comprising a matrix polymer and a complexing agent, wherein interaction of the complexing agent with the target molecule produces detectable ions; and

measuring the concentration of detectable ions.

112. A method of claim 111, comprising the additional steps of:

determining a baseline concentration of detectable ions before exposing the target molecule to the complexing agent; and

comparing the baseline concentration of detectable ions with the measured concentration of detectable ions to determine a change in ion concentration.

113. A method of claim 111 wherein the complexing agent is a boronic acid compound.

114. A method of claim 111 wherein the complexing agent is a metal complexing agent.

115. A method of claim 111 wherein the target molecule is a diol compound.

116. A method of claim 113 wherein the target molecule is a diol compound.

117. A method of claim 114 wherein the target molecule is a diol compound.

118. A method of claim 115 wherein the solution comprises a biological fluid.

119. A method of claim 113 wherein the boronic acid compound is a polymer selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

120. A method of claim 114, wherein the metal complexing agent is a copper(II) complexing agent.

121. A method of claim 114, wherein the metal complexing agent is selected from the group consisting of copper(II)-triazacyclononane; copper(II)-1,4-dimethyl-triazacyclononane; copper(II)-ethylenediamine; copper(II)-propylenediamine; copper(II)-iminodiacetate; copper(II)-diethylenetriamine; copper(II)-1-oxa-4,7-diazacyclononane; and copper(II)-1-thia-4,7-diazacyclononane.

122. A method of claim 111, wherein the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether).

123. A method of claim 122, wherein the complexing agent is a boronic acid compound.

124. A method of claim 123, wherein the boronic acid compound is a polymer selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid);

poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

125. A method of claim 111, wherein the concentration of detectable ions is measured using an electrode.

126. A method of claim 125, wherein the electrode is an interdigitated metal electrode.

127. A method of claim 111 wherein the concentration of detectable ions is measured by evaluating at least one of a change in hydrogen ion concentration, acidity, pH, conductance, and impedance.

128. A method of claim 111, wherein a semipermeable barrier is interposed between the gel and the solution.

129. A method of claim 128 wherein the semipermeable membrane is a bipolar ion-exchange membrane.

130. A method of claim 129, wherein the bipolar ion-exchange membrane has a cationic layer and an anionic layer, and the anionic layer is in contact with the gel.

131. A method for measuring the concentration of a target molecule in a solution, comprising the steps of:

exposing a target molecule to a water-insoluble hydrogel comprising a matrix polymer and a complexing agent, wherein the hydrogel is separated from the solution by a semipermeable barrier; and

measuring the concentration of detectable ions produced by interaction of the complexing agent with the target molecule.

132. A method of claim 131 wherein the concentration of detectable ions is measured by evaluating at least one of a change in hydrogen ion concentration, acidity, pH, conductance, and impedance.

133. A method of claim 131 wherein the concentration of detectable ions is measured using an electrode.

134. A method of claim 133 wherein the electrode is an interdigitated metal electrode.

135. A method of claim 131 wherein the complexing agent is a boronic acid compound.

136. A method of claim 131, wherein the boronic acid compound is a polymer selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

137. A method of claim 131 wherein the complexing agent is a metal complexing agent.

138. A method of claim 137, wherein the metal complexing agent is selected from the group consisting of copper(II)-triazacyclononane; copper(II)-1,4-dimethyl-triazacyclononane; copper(II)-ethylenediamine; copper(II)-propylenediamine; copper(II)-iminodiacetate; copper(II)-diethylenetriamine; copper(II)-1-oxa-4,7-diazacyclononane; and copper(II)-1-thia-4,7-diazacyclononane.

139. A method of claim 131, wherein the matrix polymer is selected from the group consisting of poly(hydroxyethyl methacrylate); poly(acrylamide); poly(hydroxypropyl methacrylate); poly(ethylene glycol), poly (ethylene glycol/co-propylene glycol), poly(N,N dimethylacrylamide), poly(vinylpyrrolidone), and poly(vinylmethyl ether).

140. A method of claim 139, wherein the complexing agent is a polymeric boronic acid compound selected from the group consisting of poly(vinyl phenyl boronic acid); poly(vinyl 3-nitrophenylboronic acid); poly(vinyl 3-methoxyphenylboronic acid); poly(vinyl 3-carboxyphenylboronic acid); poly(vinyl 5-bromophenylboronic acid); poly(vinyl 3-methylphenylboronic acid); and poly(vinyl 3,5-dichlorophenylboronic acid).

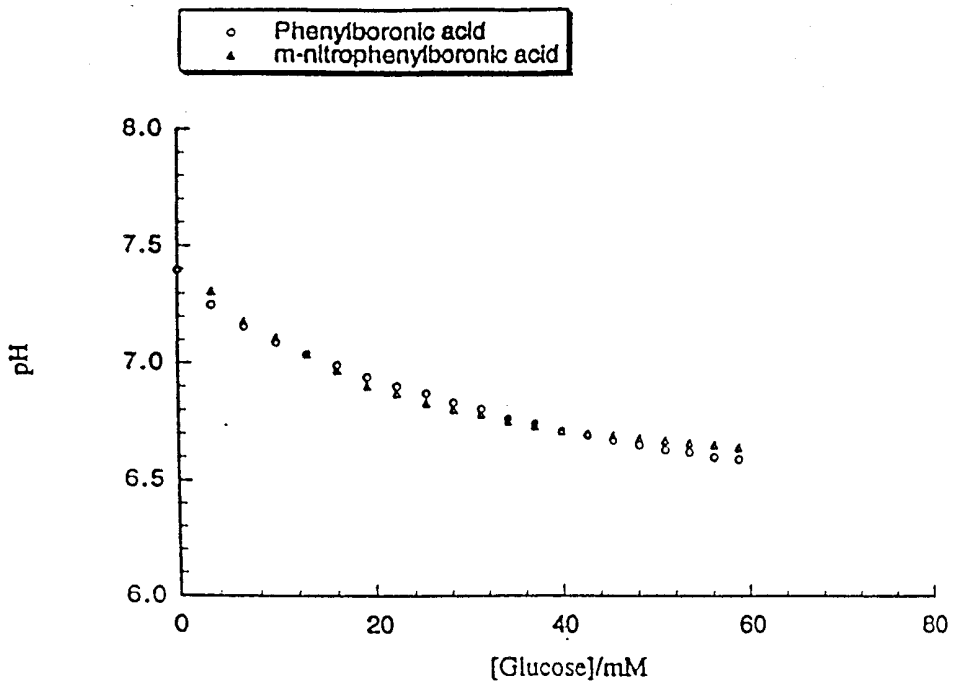

Phenylboronic acid
m-nitrophenylboronic acid
8.0
7.5
7.0
6.5
6.0
pH
0
20
40
60
80
[Glucose]/mM

FIG. 1

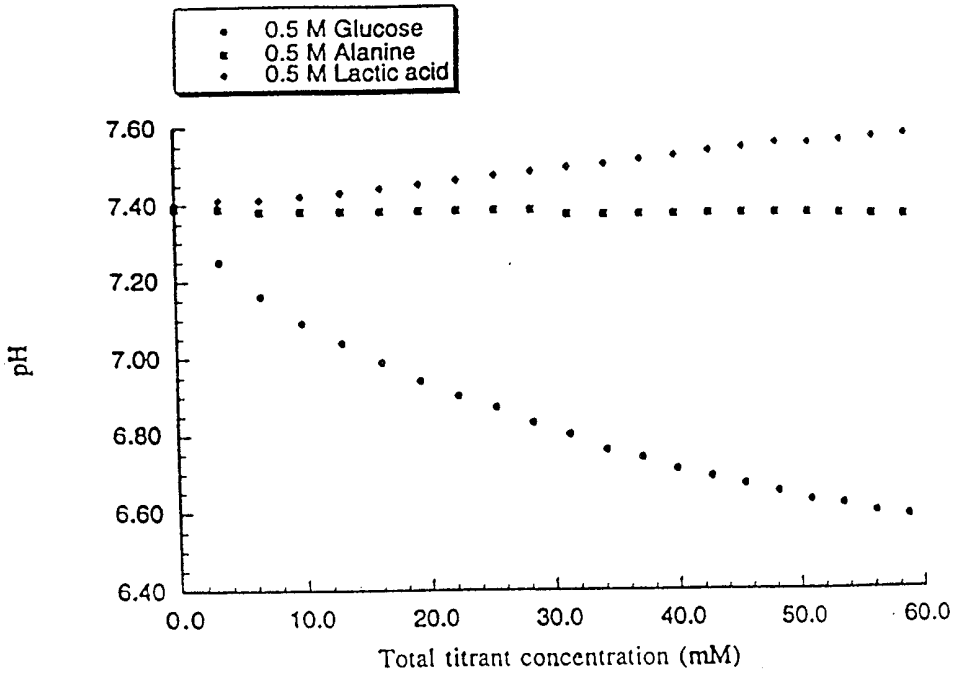

0.5 M Glucose
0.5 M Alanine
0.5 M Lactic acid
7.60
7.40
7.20
7.00
6.80
6.60
6.40
pH
0.0
10.0
20.0
30.0
40.0
50.0
60.0
Total titrant concentration (mM)

FIG. 2

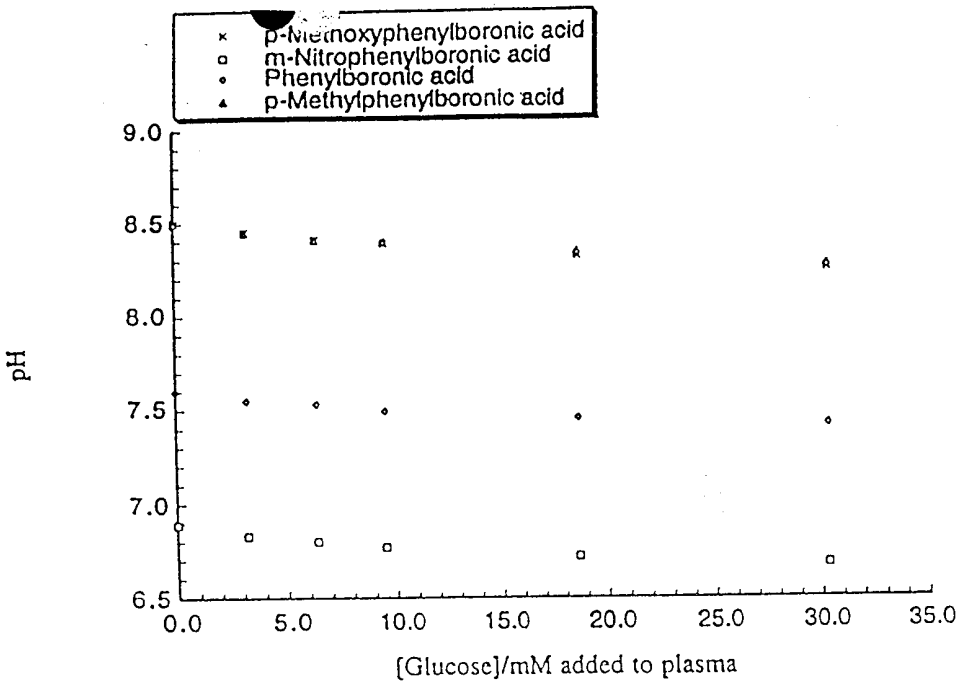

p-Methoxyphenylboronic acid
m-Nitrophenylboronic acid
Phenylboronic acid
p-Methylphenylboronic acid
pH
9.0
8.5
8.0
7.5
7.0
6.5
0.0
5.0
10.0
15.0
20.0
25.0
30.0
35.0
[Glucose]/mM added to plasma

FIG. 3

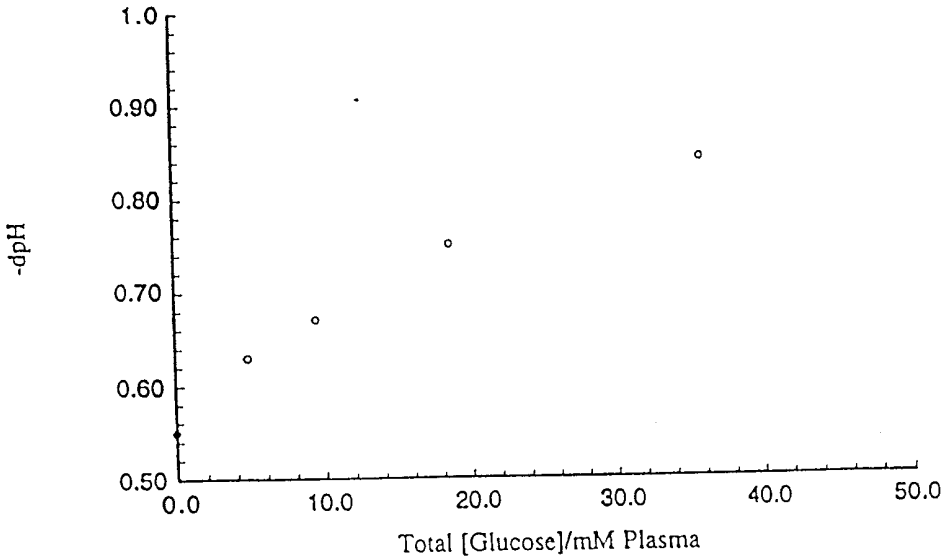

-dpH
1.0
0.90
0.80
0.70
0.60
0.50
0.0
10.0
20.0
30.0
40.0
50.0
Total [Glucose]/mM Plasma

FIG. 4

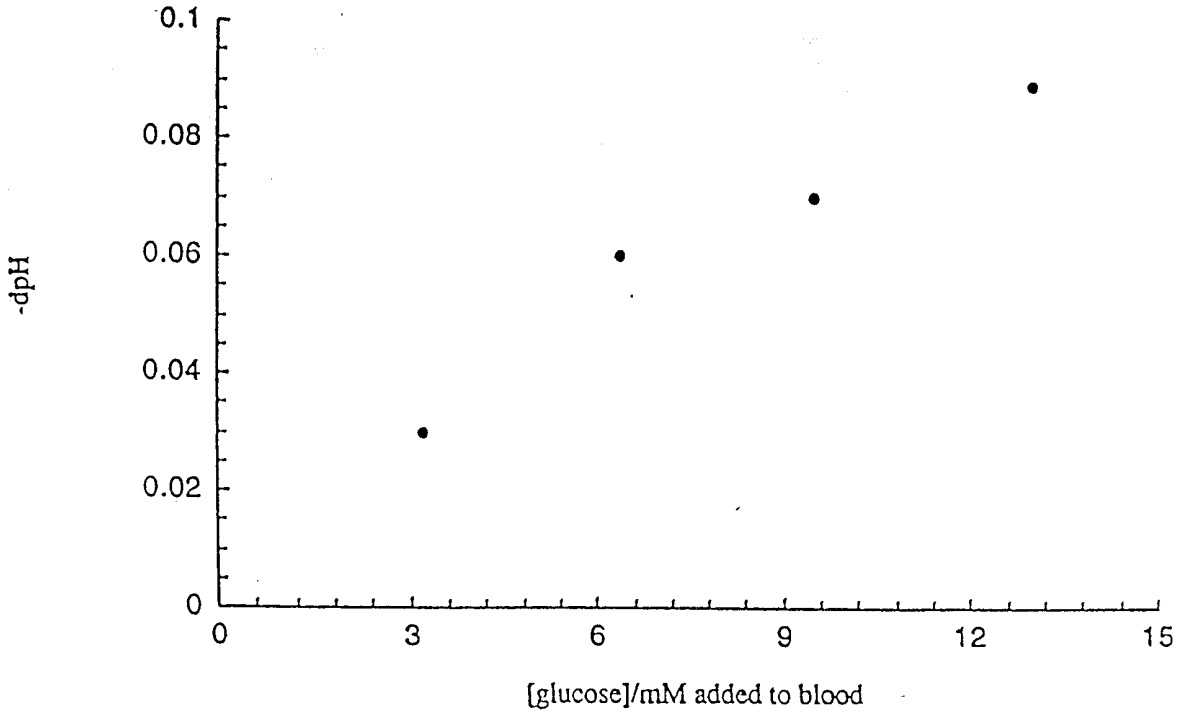

0.1
0.08
0.06
0.04
0.02
0
-dpH
0
3
6
9
12
15
[glucose]/mM added to blood

FIG. 5

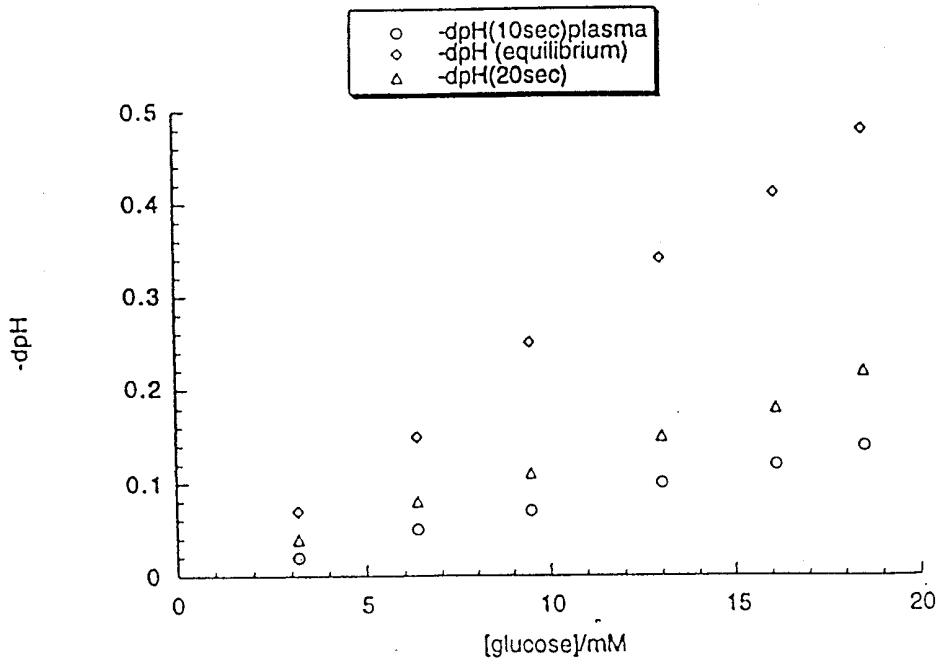

-dpH(10sec)plasma
-dpH (equilibrium)
-dpH(20sec)
0.5
0.4
0.3
0.2
0.1
0
-dpH
0
5
10
15
20
[glucose]/mM

FIG. 6

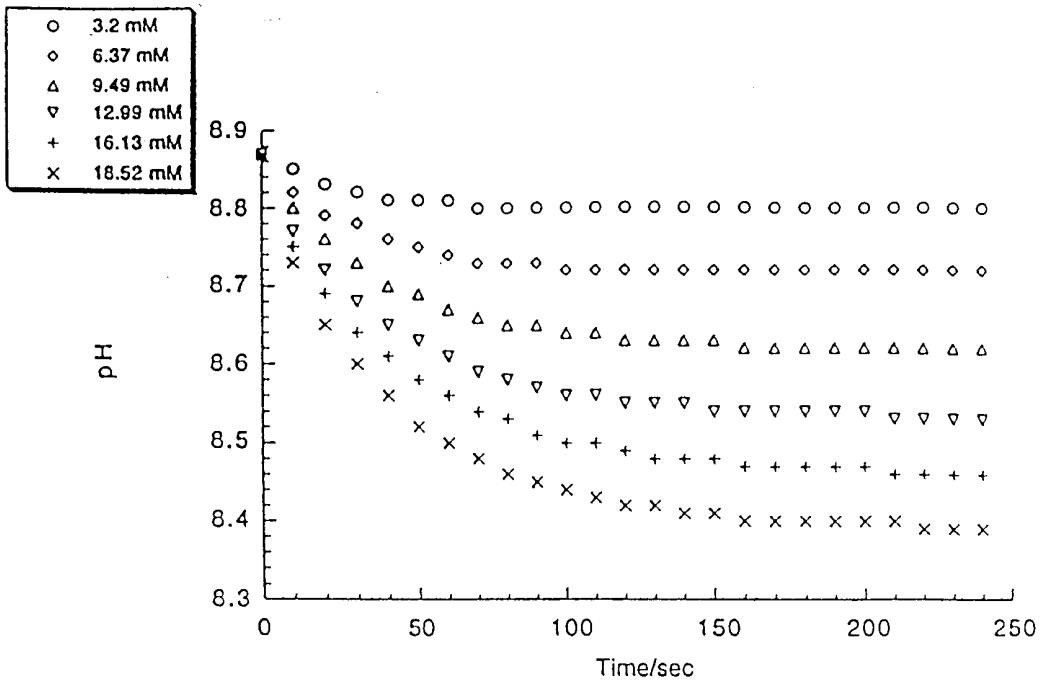

3.2 mM
6.37 mM
9.49 mM
12.99 mM
16.13 mM
18.52 mM
pH
8.9
8.8
8.7
8.6
8.5
8.4
8.3
0
50
100
150
200
250
Time/sec

FIG. 7

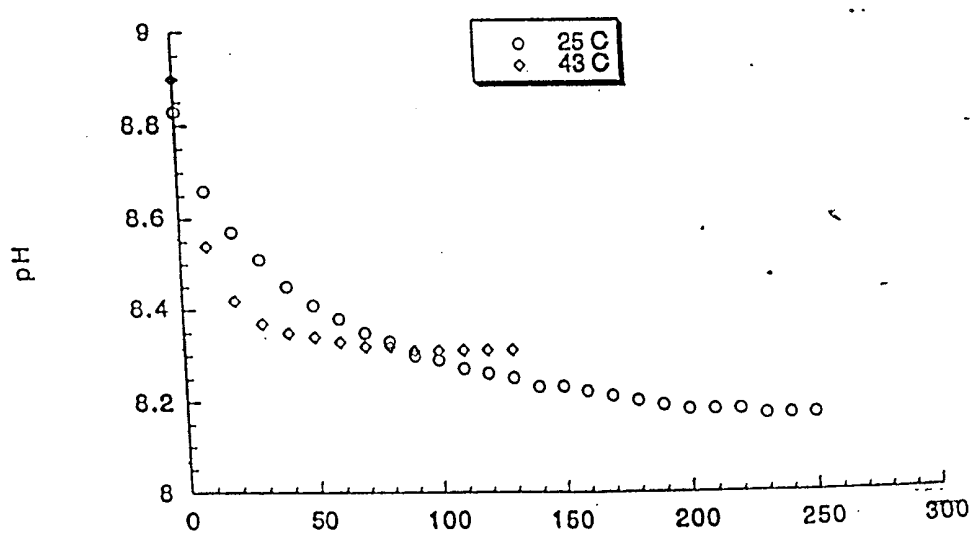

25 C
43 C
pH
9
8.8
8.6
8.4
8.2
8
0
50
100
150
200
250
300

FIG. 8

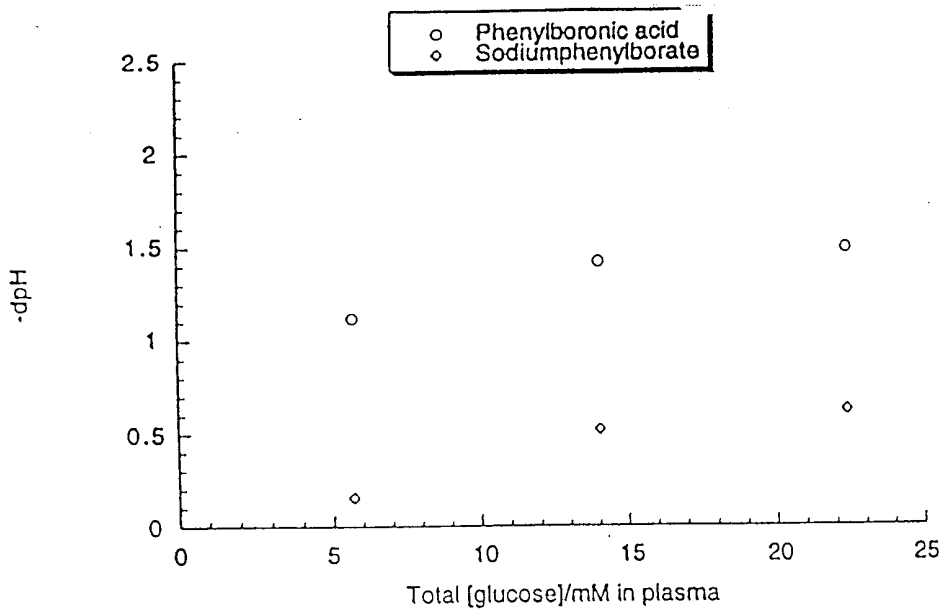

Phenylboronic acid
Sodiumphenylborate
-dpH
2.5
2
1.5
1
0.5
0
0
5
10
15
20
25
Total [glucose]/mM in plasma

FIG. 9

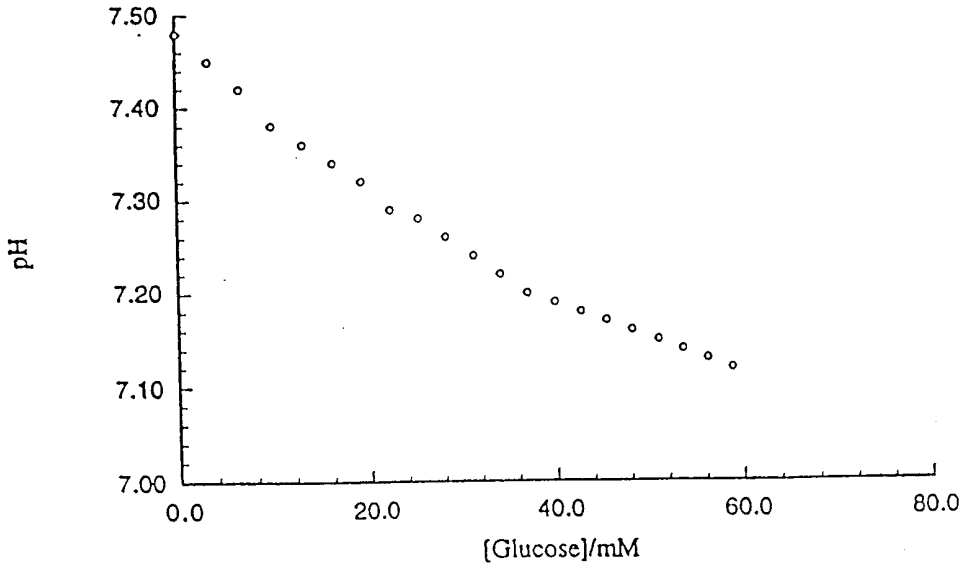

pH
7.50
7.40
7.30
7.20
7.10
7.00
0.0
20.0
40.0
60.0
80.0
[Glucose]/mM

FIG. 10

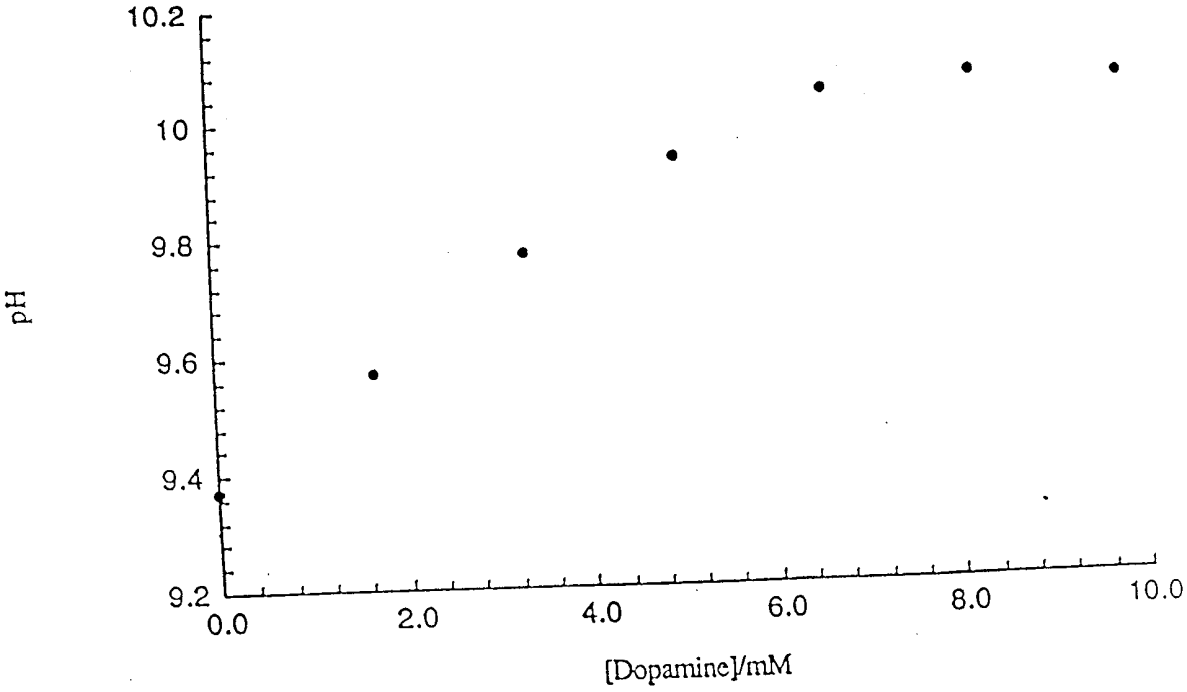

10.2
10
9.8
9.6
9.4
9.2
pH
0.0
2.0
4.0
6.0
8.0
10.0
[Dopamine]/mM

FIG. 11

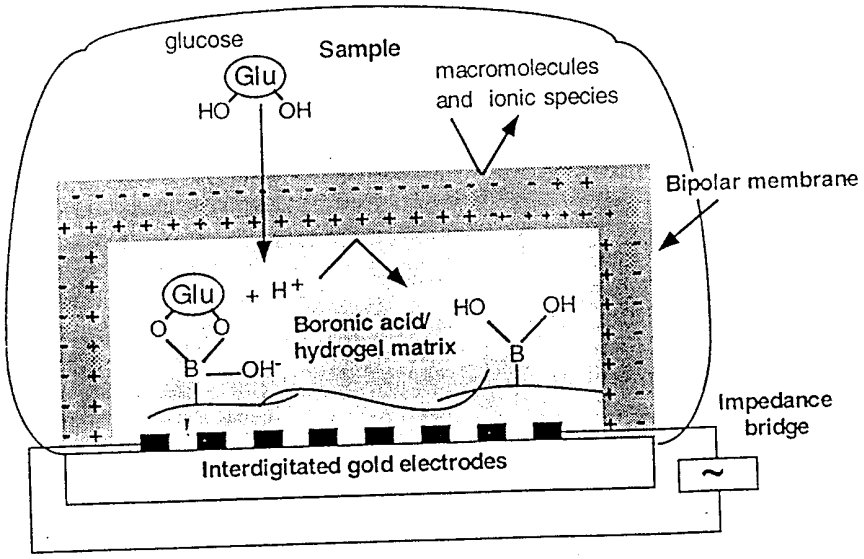

glucose
Sample
Glu
HO
OH
macromolecules
and ionic species
Bipolar membrane
Glu
+ H+
Boronic acid/
hydrogel matrix
HO
OH
B
OH-
Impedance
bridge
Interdigitated gold electrodes
~

FIG. 12

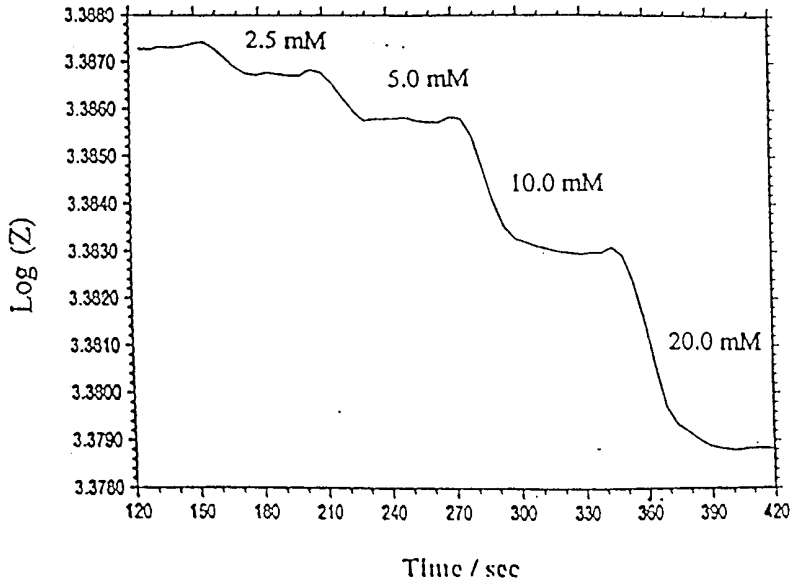


FIG. 13A

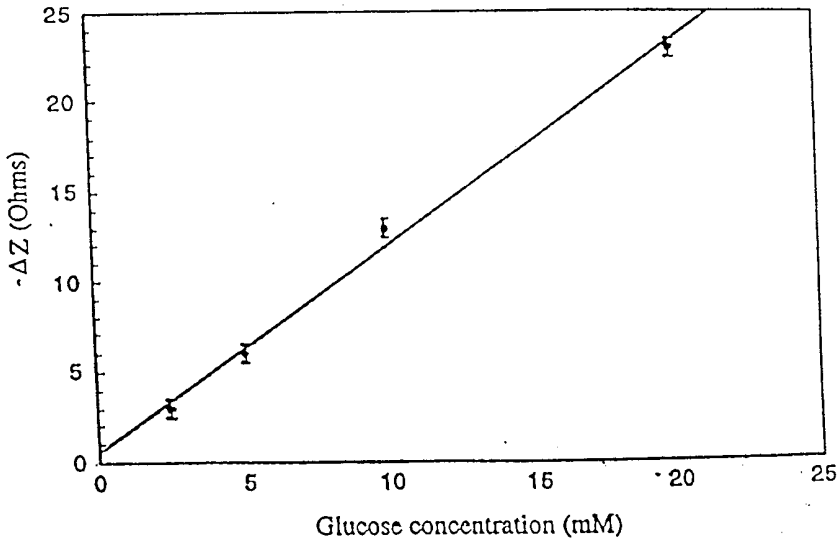


FIG. 13B

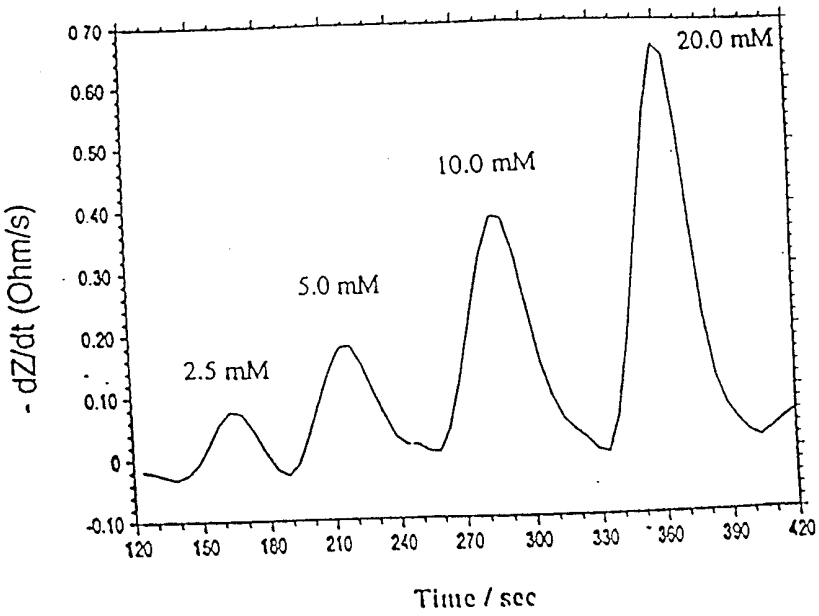


FIG. 13C

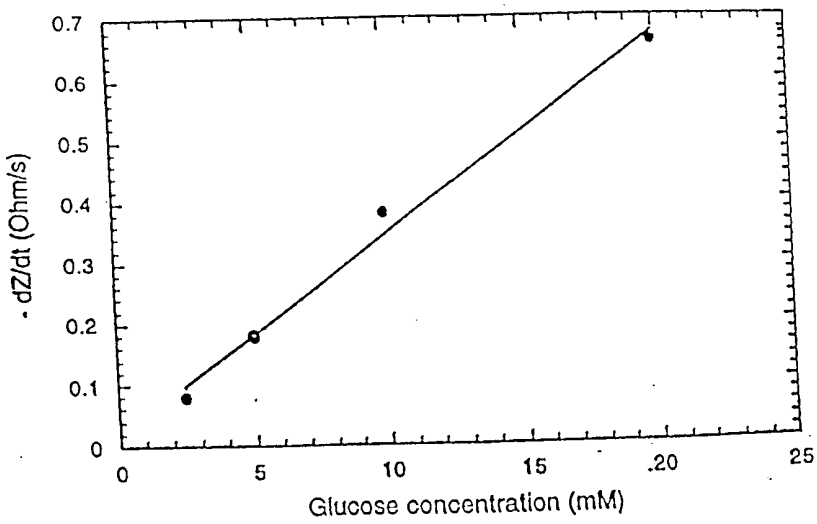


FIG. 13D

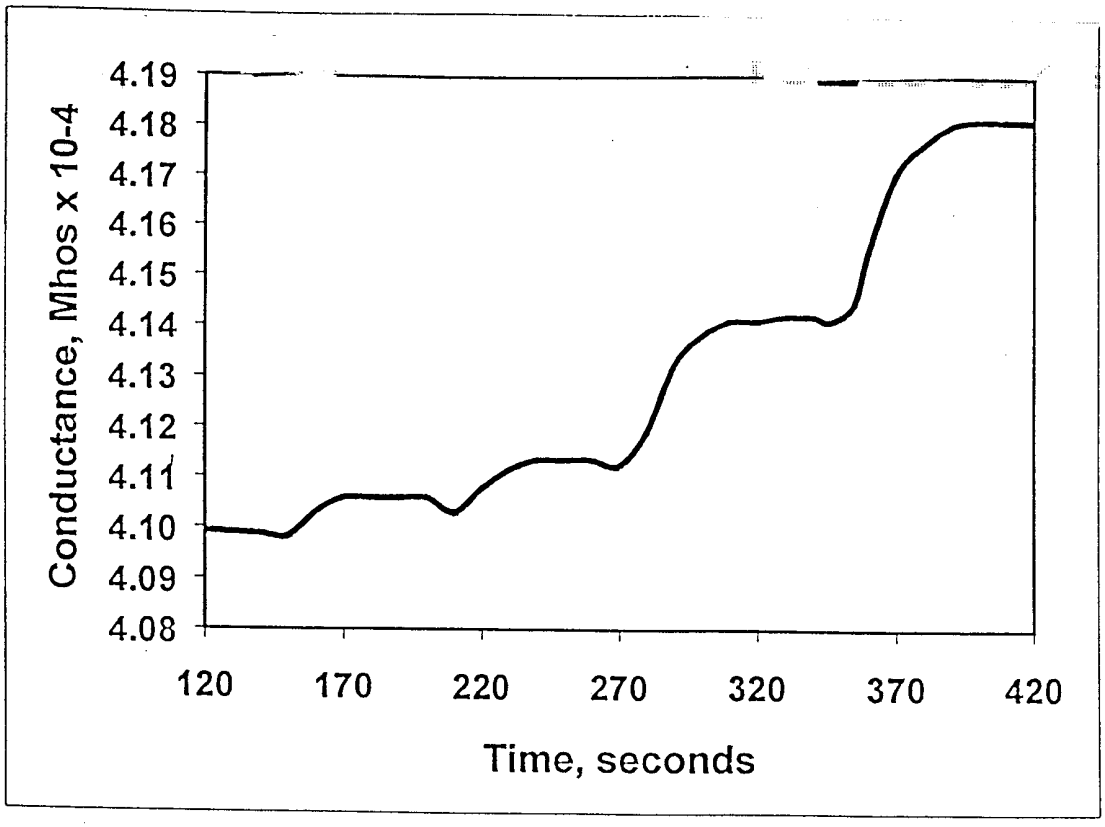


FIG. 13E

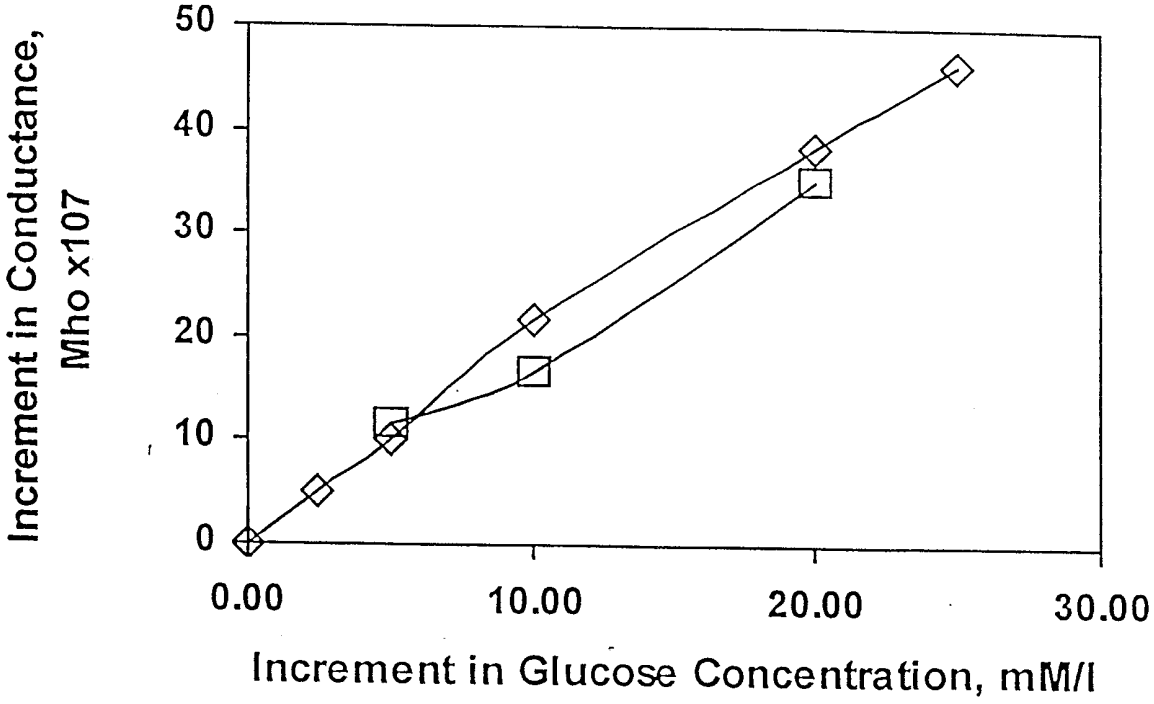


FIG. 13F

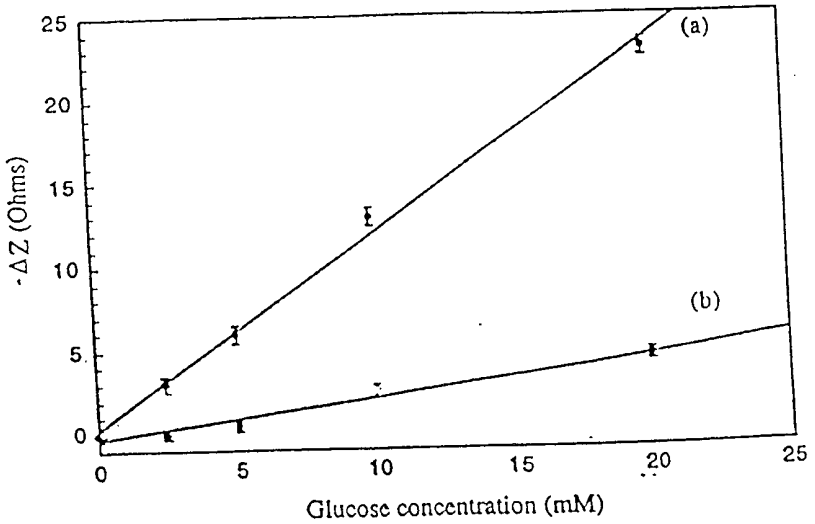

25
20
15
10
5
0
-ΔZ (Ohms)
(a)
(b)
0
5
10
15
20
25
Glucose concentration (mM)

FIG. 14

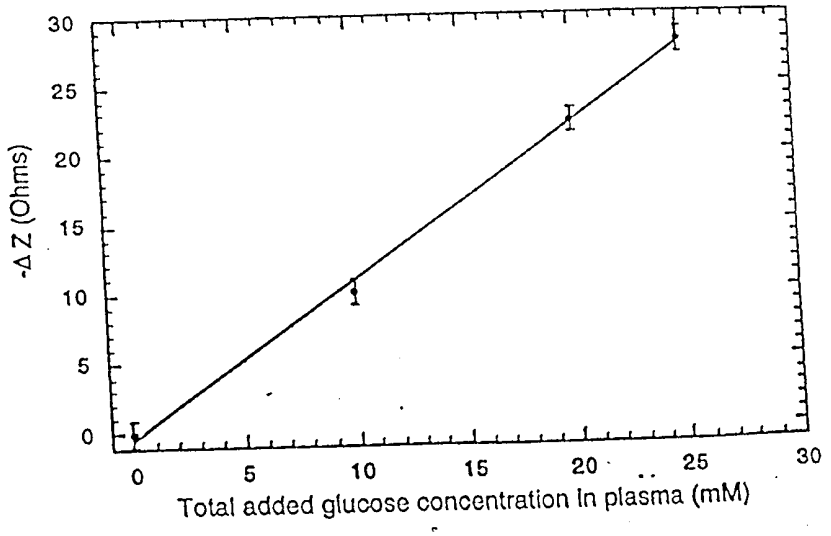

30
25
20
15
10
5
0
-ΔZ (Ohms)
0
5
10
15
20
25
30
Total added glucose concentration in plasma (mM)

FIG. 15

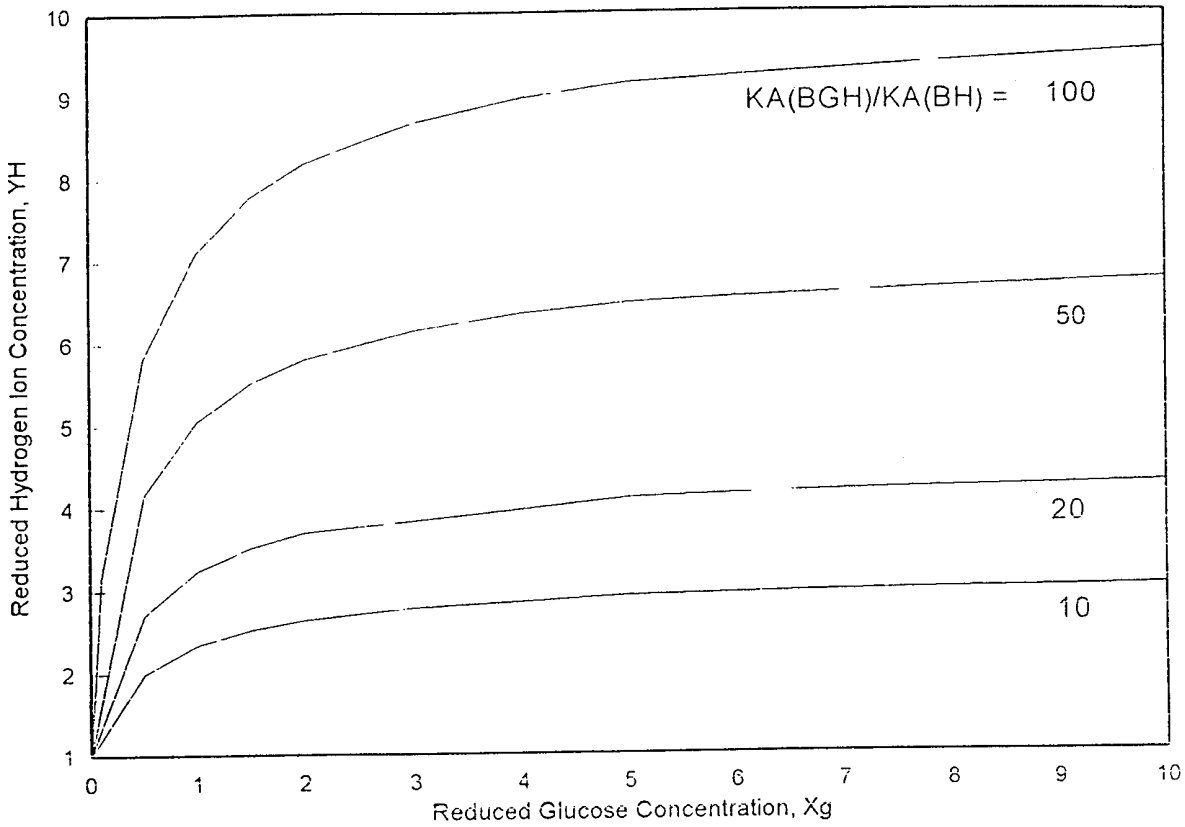

Reduced Hydrogen Ion Concentration, YH
Reduced Glucose Concentration, Xg
KA(BGH)/KA(BH) = 100
50
20
10
1
2
3
4
5
6
7
8
9
10
0
1
2
3
4
5
6
7
8
9
10

FIG. 16

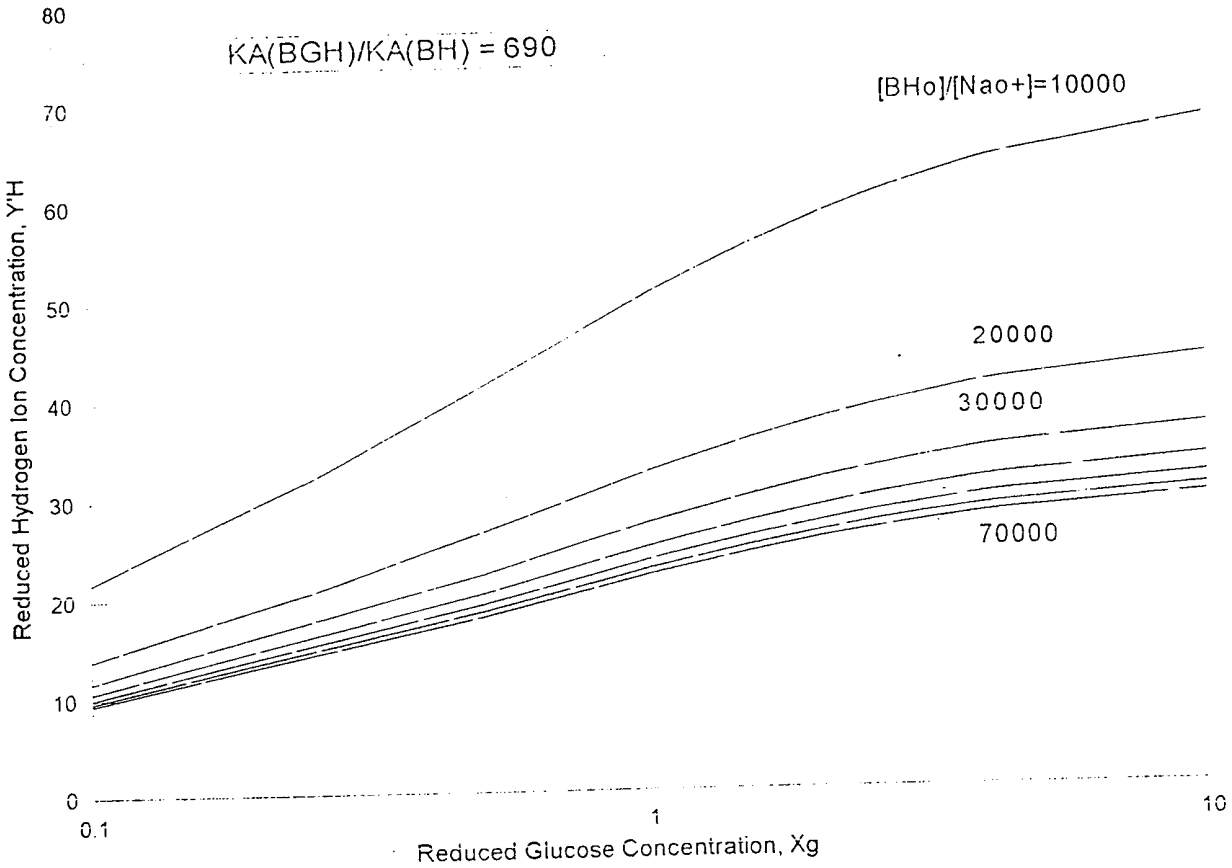

KA(BGH)/KA(BH) = 690
[BHo]/[Nao+]=10000
20000
30000
70000
Reduced Hydrogen Ion Concentration, Y'H
80
70
60
50
40
30
20
10
0
0.1
1
10
Reduced Glucose Concentration, Xg

FIG. 17

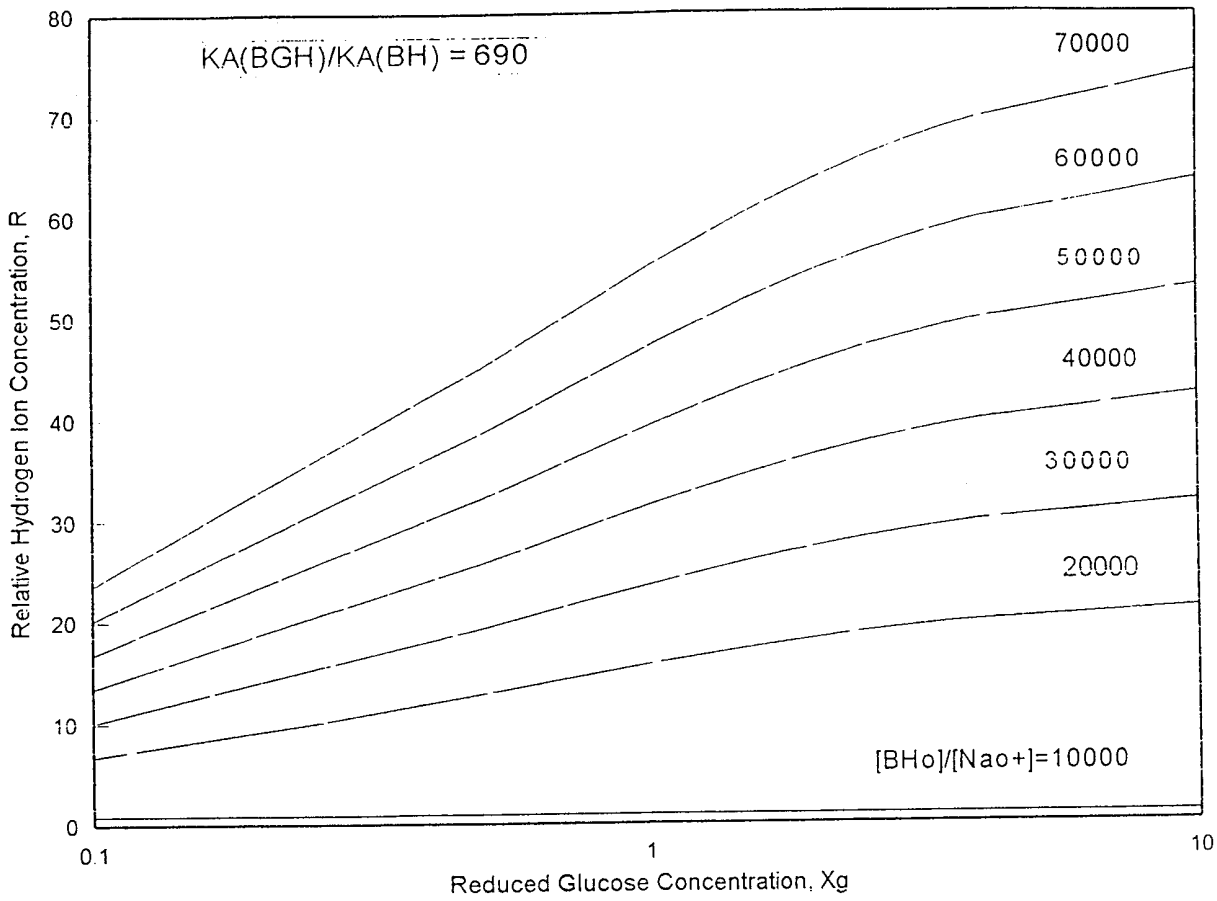

KA(BGH)/KA(BH) = 690
70000
60000
50000
40000
30000
20000
[BHo]/[Nao+]=10000
Relative Hydrogen Ion Concentration, R
Reduced Glucose Concentration, Xg
0
10
20
30
40
50
60
70
80
0.1
1
10

FIG. 18

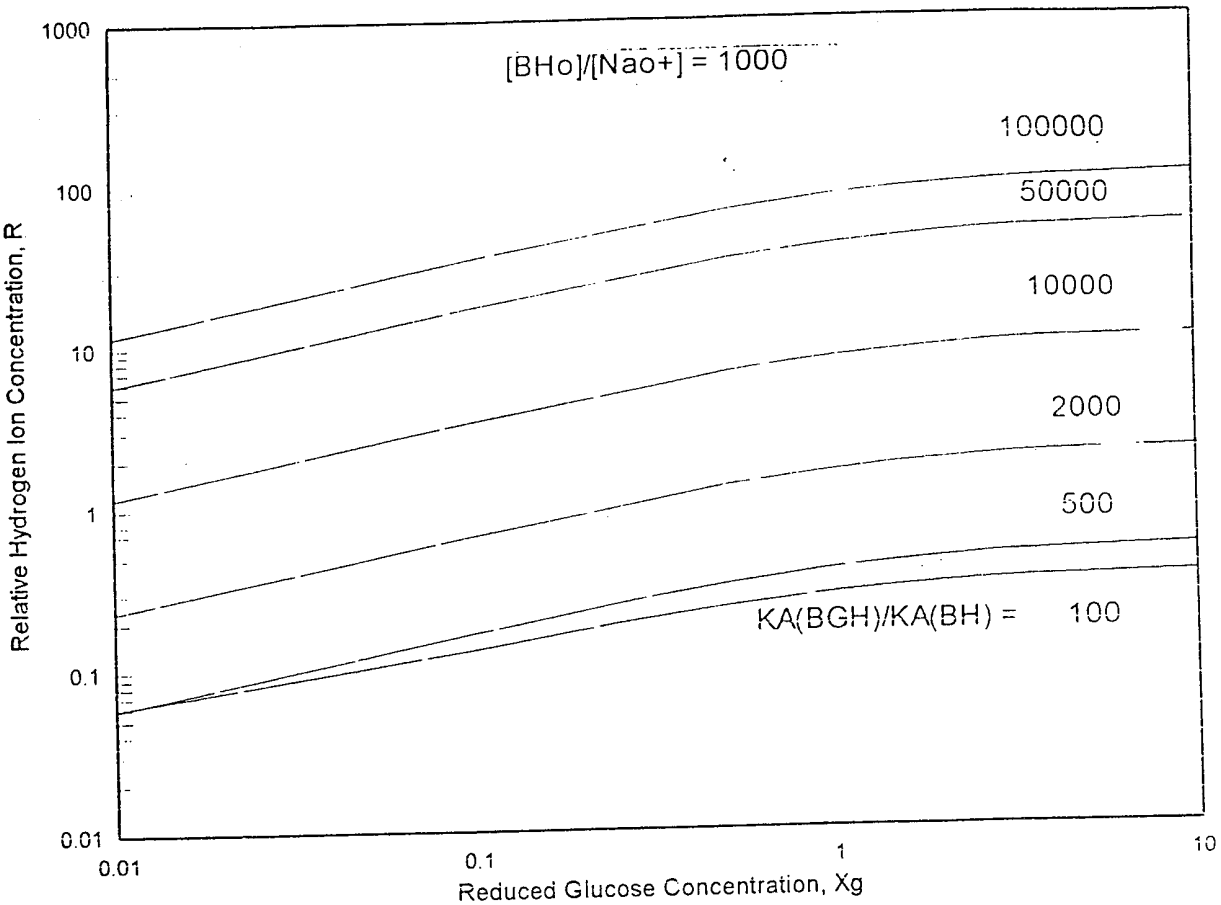

[BHo]/[Nao+] = 1000
100000
50000
10000
2000
500
KA(BGH)/KA(BH) = 100
Relative Hydrogen Ion Concentration, R
1000
100
10
1
0.1
0.01
0.01
0.1
1
10
Reduced Glucose Concentration, Xg

FIG. 19