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Van Antwerp et al.

(10) **Patent No.: US 6,784,274 B2**
(45) **Date of Patent: Aug. 31, 2004**

(54) **HYDROPHILIC, SWELLABLE COATINGS FOR BIOSENSORS**

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(73) Assignee: **Minimed Inc.**, Sylmar, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 44 days.

(21) Appl. No.: **10/213,265**

(22) Filed: **Aug. 6, 2002**

(65) **Prior Publication Data**

US 2003/0069383 A1 Apr. 10, 2003

Related U.S. Application Data

(63) Continuation of application No. 09/123,930, filed on Jul. 28, 1998, now Pat. No. 6,462,162, which is a continuation of application No. 08/749,754, filed on Oct. 24, 1996, now Pat. No. 5,786,439.

(51) **Int. Cl.**[7] **C08G 18/52**

(52) **U.S. Cl.** **528/77**; 427/2.12; 427/58; 428/423.1; 428/426; 528/85

(58) **Field of Search** 427/2.12, 58; 428/423.1, 428/426; 528/77, 85

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EP	0 624 612 A1	11/1994
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GB	2235462	3/1991
WO	WO 92/21976	12/1992
WO	WO 95/15352	6/1995
WO	WO 96/15443	5/1996
WO	WO 96/30431	10/1996

* cited by examiner

Primary Examiner—John M. Cooney, Jr.
(74) *Attorney, Agent, or Firm*—Gates & Cooper LLP

(57) **ABSTRACT**

Methods for reducing the electrode impedance of implantable biosensors by coating the surface of the biosensor with a uniform hydrogel which allows unimpeded water movement around the sensor are provided. The surface coatings are compositions which are biocompatible and are capable of water uptake of at least 120% of their weight, more preferably at least 200% of their weight. Upon the uptake of water, the hydrogels used in the present invention will also swell and provide a layer of water around the electrodes to which the hydrogels are attached. The hydrogels can be prepared from (a) a diisocyanate, (b) a hydrophilic polymer which is a hydrophilic diol, a hydrophilic diamine, or a combination thereof, and optionally, (c) a chain extender.

19 Claims, 14 Drawing Sheets

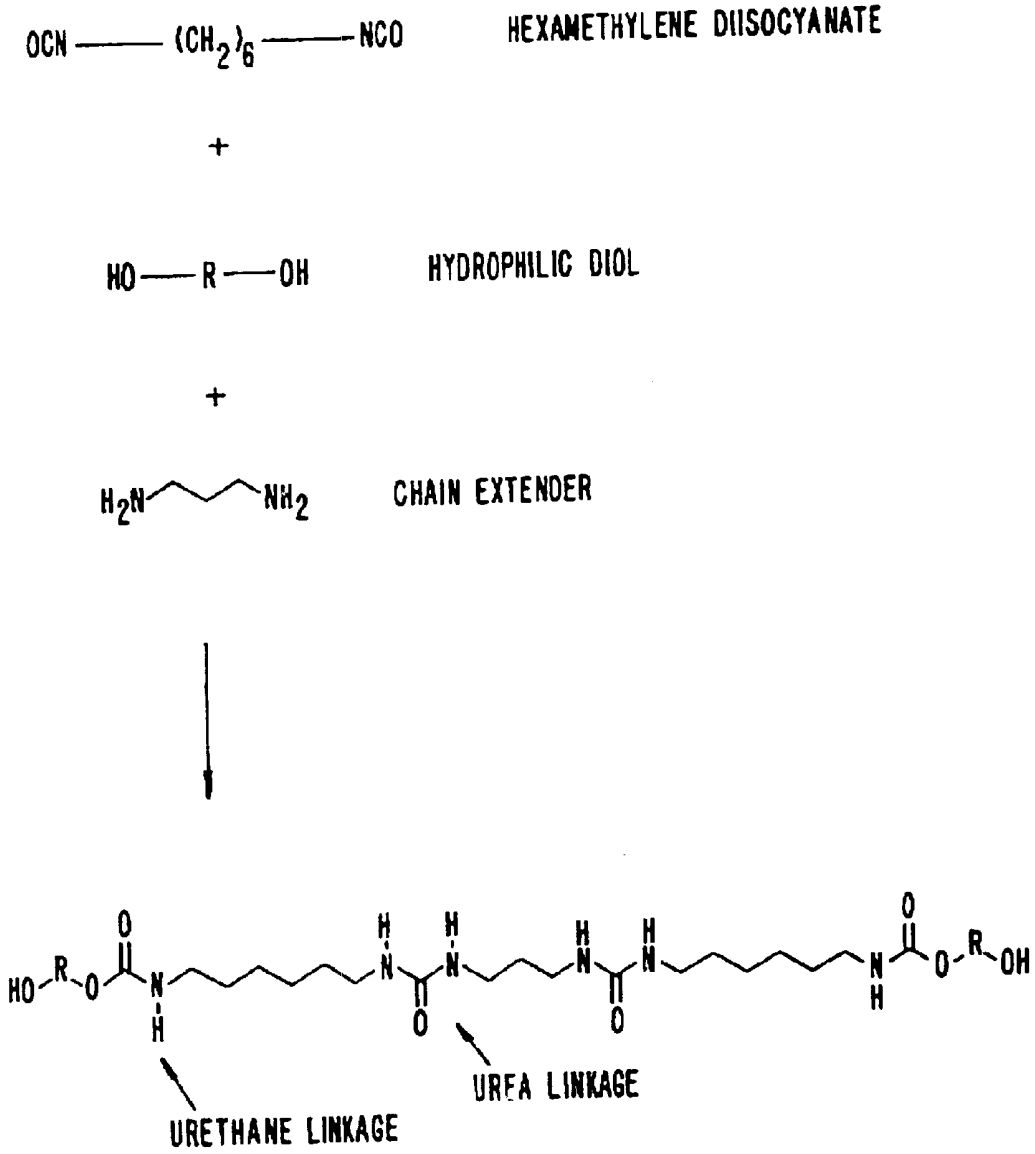

OCN —— (CH2)6 —— NCO
HEXAMETHYLENE DIISOCYANATE
+
HO — R — OH
HYDROPHILIC DIOL
+
H2N NH2
CHAIN EXTENDER
HO R O
URETHANE LINKAGE
UREA LINKAGE
O R OH

FIG. 1A.

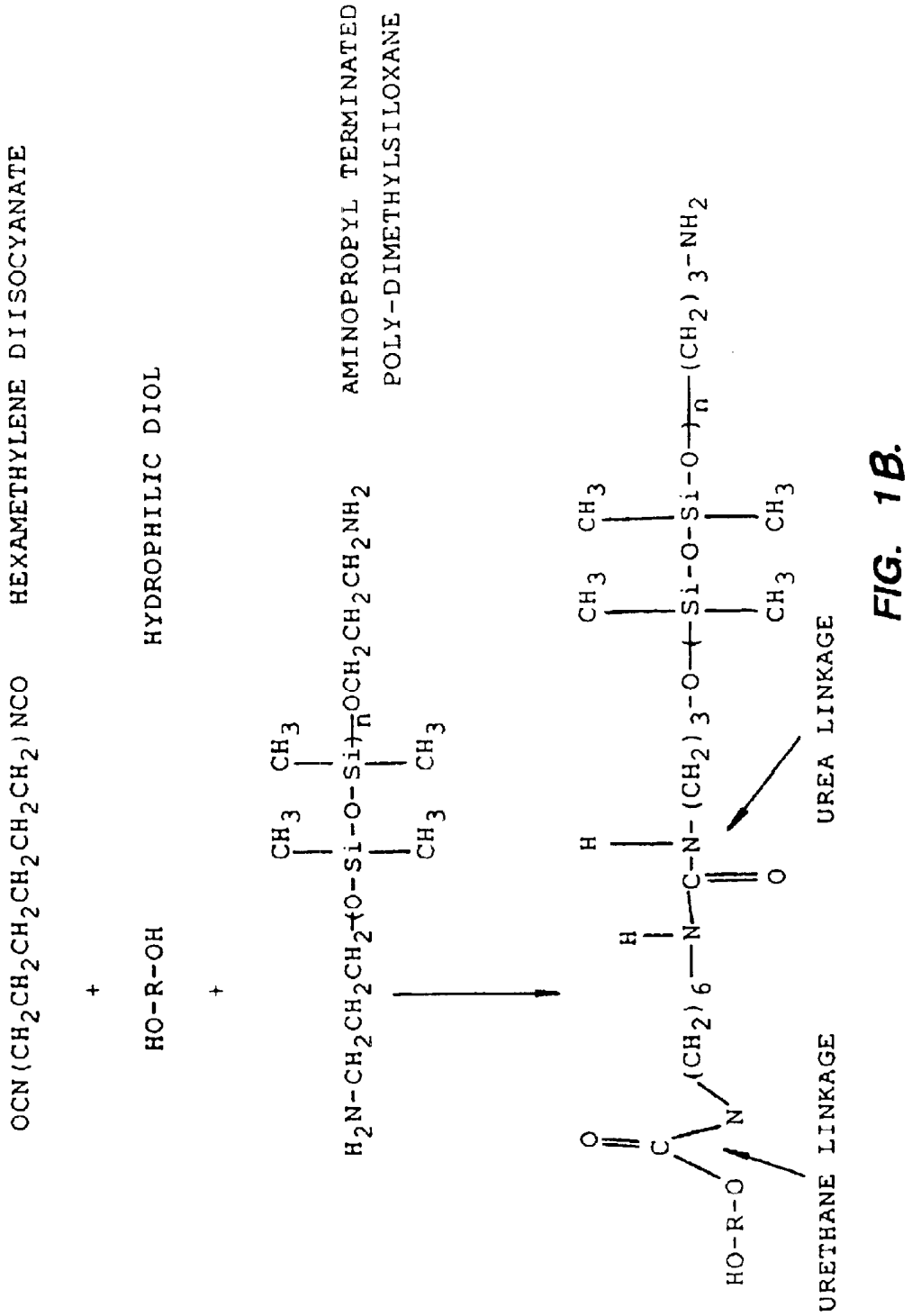
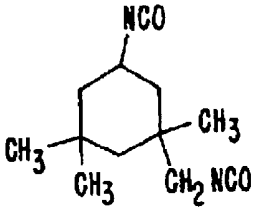
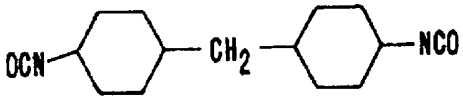

OCN(CH2CH2CH2CH2CH2CH2)NCO
HEXAMETHYLENE DIISOCYANATE
+
HO-R-OH
HYDROPHILIC DIOL
+
H2N-CH2CH2CH2-Si-O-Si-O-Si-OCH2CH2CH2NH2
AMINOPROPYL TERMINATED
POLY-DIMETHYLSILOXANE
URETHANE LINKAGE
UREA LINKAGE

FIG. 1B.

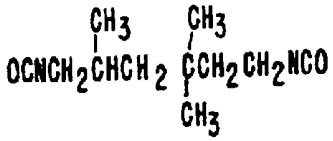
HEXAMETHYLENE DIISOCYANATE
HMI



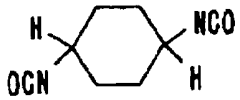
ISOPHORONE DIISOCYANATE
IPDI



4,4'-DICYCLOHEXYLMETHANEDILSOCYANATE
HI2MDI



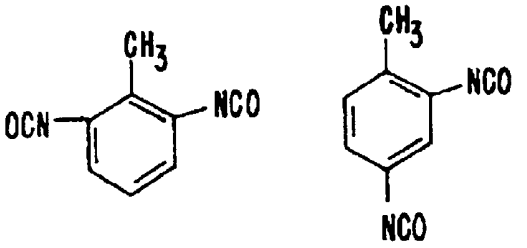
TRIMETHYLHEXAMETHYLENE DIISOCYANATE
TMDI



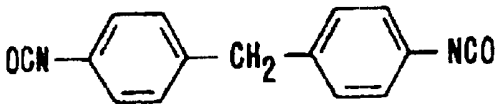
TRANS-1,4-CYCLOHEXANE DIISOCYANATE
CHDI

1,3-BIS(ISOCYANTOMETHYL)CYCLOHEXANE
CIS AND TRANS

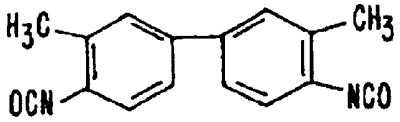
FIG. 2.



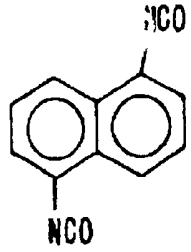
TOLUENEDIISOCYANATE
TDI



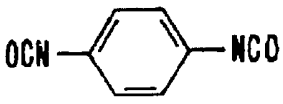
4,4'-DIPHENYLMETHANE DIISOCYANATE
MDI



3,3'-DIMETHYL-4,4'-BIPHENYL
DIISOCYANATE TODI



NAPHTAHALENE DIISOCYANATE
NDI



PARAPHENYLENE DIISOCYANATE
PPDI

FIG. 3.

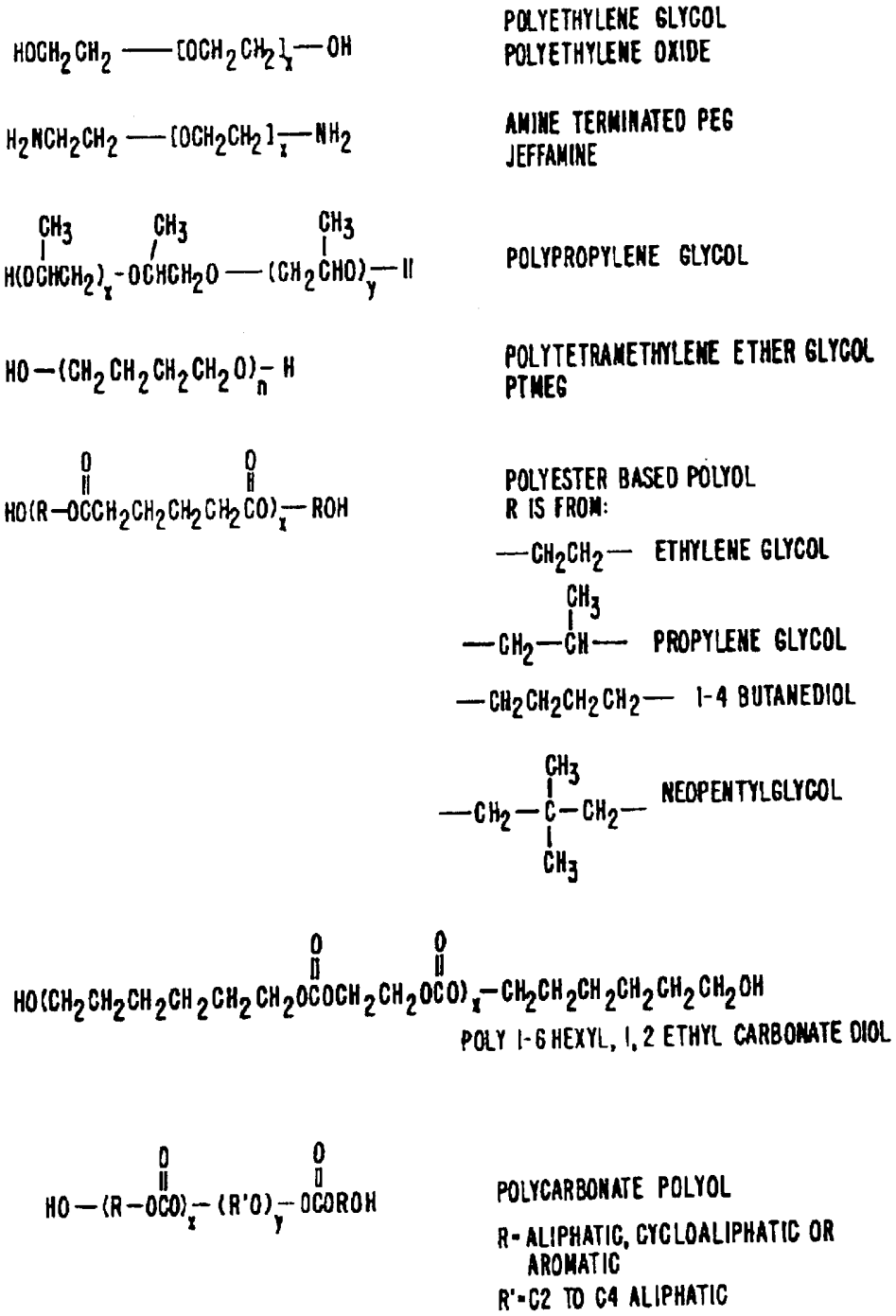

POLYETHYLENE GLYCOL
POLYETHYLENE OXIDE
AMINE TERMINATED PEG
JEFFAMINE
POLYPROPYLENE GLYCOL
POLYTETRAMETHYLENE ETHER GLYCOL
PTMEG
POLYESTER BASED POLYOL
R IS FROM:
ETHYLENE GLYCOL
PROPYLENE GLYCOL
1-4 BUTANEDIOL
NEOPENTYLGLYCOL
POLY 1-6 HEXYL, 1, 2 ETHYL CARBONATE DIOL
POLYCARBONATE POLYOL
R-ALIPHATIC, CYCLOALIPHATIC OR AROMATIC
R'-C2 TO C4 ALIPHATIC

FIG 4.

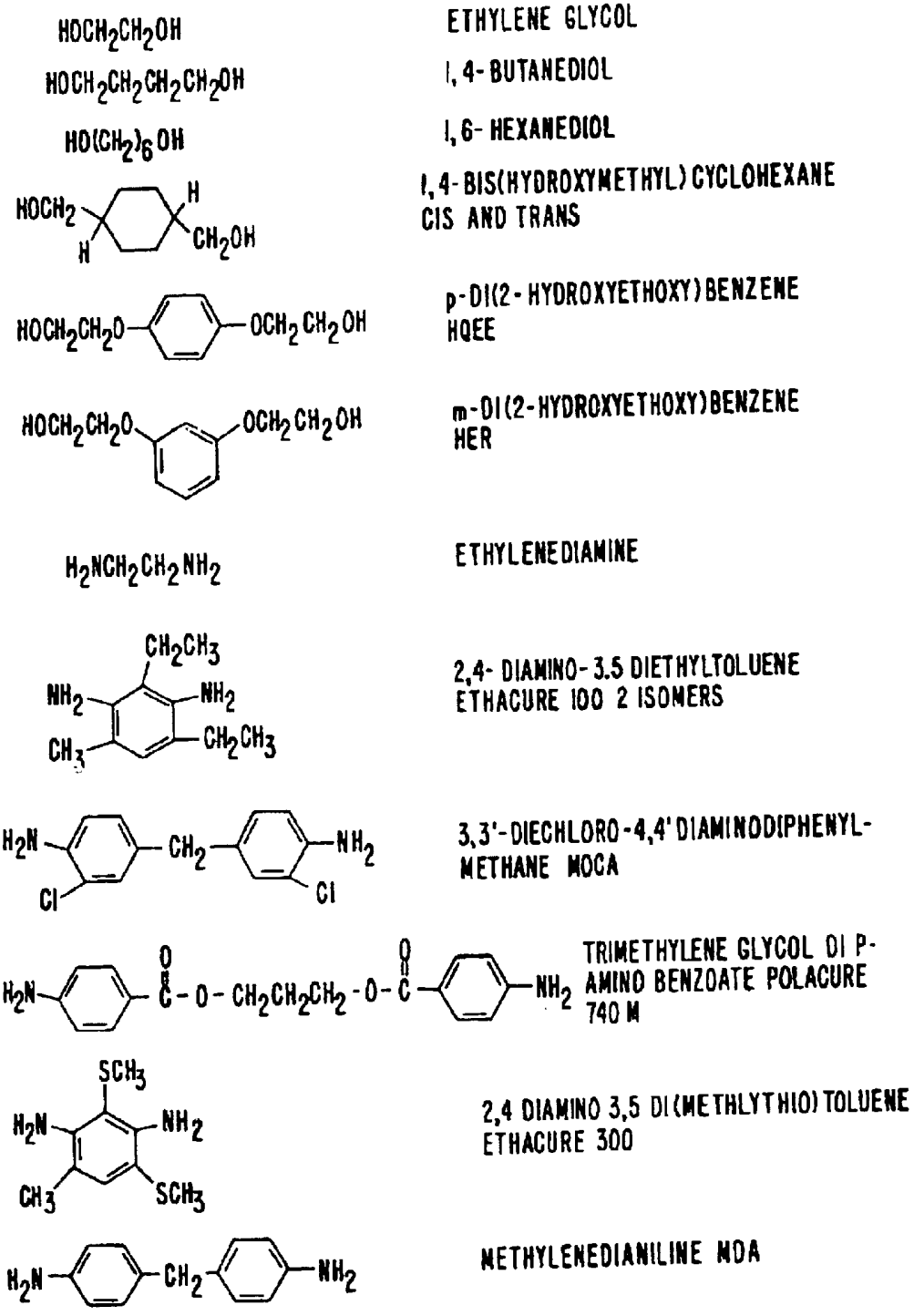
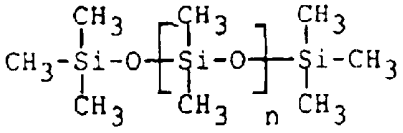
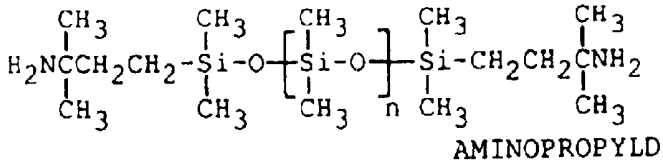

HOCH2CH2OH
ETHYLENE GLYCOL
HOCH2CH2CH2CH2OH
1,4-BUTANEDIOL
HO(CH2)6OH
1,6-HEXANEDIOL
HOCH2
H
CH2OH
H
1,4-BIS(HYDROXYMETHYL) CYCLOHEXANE CIS AND TRANS
HOCH2CH2O
OCH2CH2OH
p-DI(2-HYDROXYETHOXY)BENZENE HQEE
HOCH2CH2O
OCH2CH2OH
m-DI(2-HYDROXYETHOXY)BENZENE HER
H2NCH2CH2NH2
ETHYLENEDIAMINE
CH2CH3
NH2
NH2
CH3
CH2CH3
2,4-DIAMINO-3.5 DIETHYLTOLUENE ETHACURE 100 2 ISOMERS
H2N
CH2
NH2
Cl
Cl
3,3'-DICHLORO-4,4'DIAMINODIPHENYL-METHANE MOCA
H2N
O
C-O-CH2CH2CH2-O-C
O
NH2
TRIMETHYLENE GLYCOL DI P-AMINO BENZOATE POLACURE 740 M
SCH3
H2N
NH2
CH3
SCH3
2,4 DIAMINO 3,5 DI(METHLYTHIO) TOLUENE ETHACURE 300
H2N
CH2
NH2
METHYLENEDIANILINE MDA

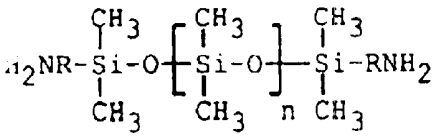
FIG. 5A.



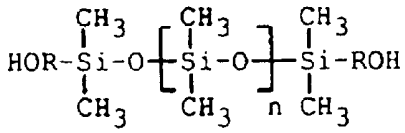
POLYDIMETHYLSILOXANE PDMS



AMINOPROPYLDIMETHYL TERMINATED PDMS



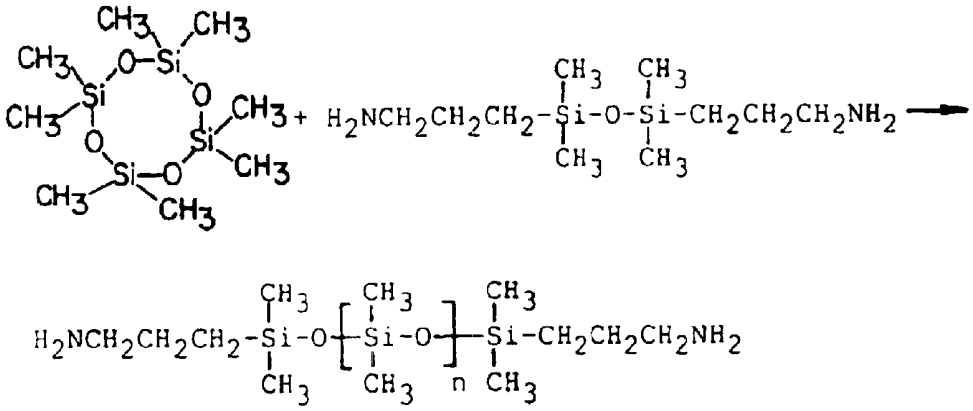
GENERAL DIFUNCTIONAL AMINE TERMINATED SILICONE
R=ALKYL, CYCLOALKYL, AROMATIC



GENERAL DIFUNCTIONAL OH TERMINATED SILICONE
R=ALKYL, CYCLOALKYL, AROMATIC

FIG. 5B.

SYNTHETIC ROUTE TO AMINO TERMINATED SILICONE POLYMER.



SYNTHETIC ROUTE TO OH TERMINATED SILICONE POLYMER

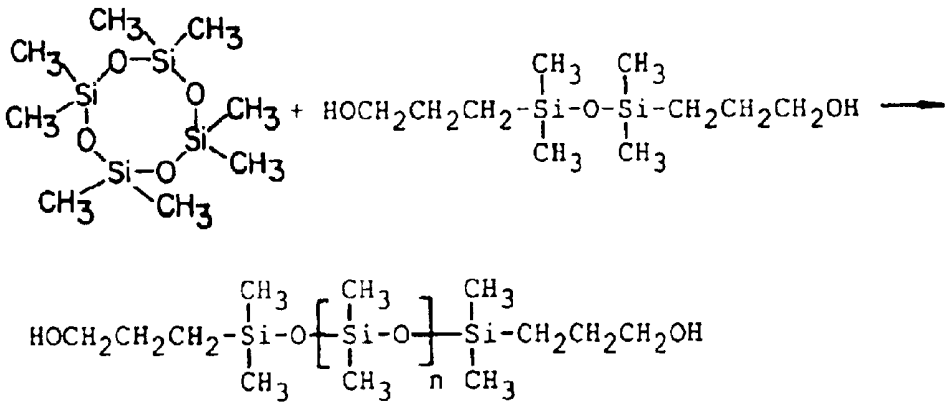
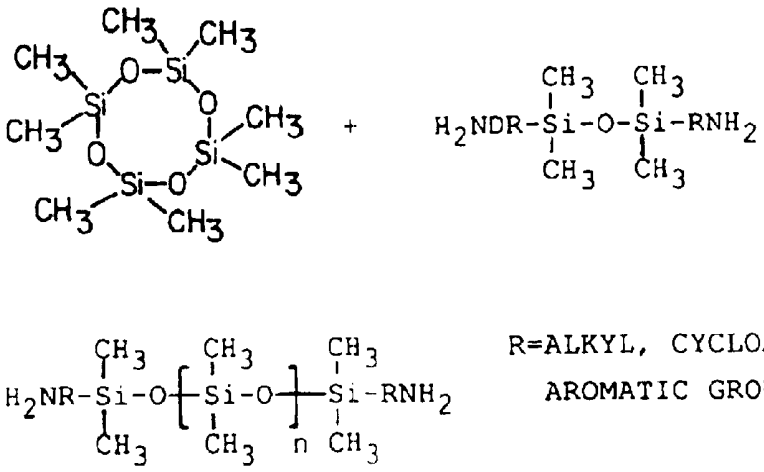


FIG. 6.

GENERAL SYNTHETIC ROUTE TO AMINO TERMINATED SILICONE POLYMER



GENERAL SYNTHETIC ROUTE TO OH TERMINATED SILICONE POLYMER

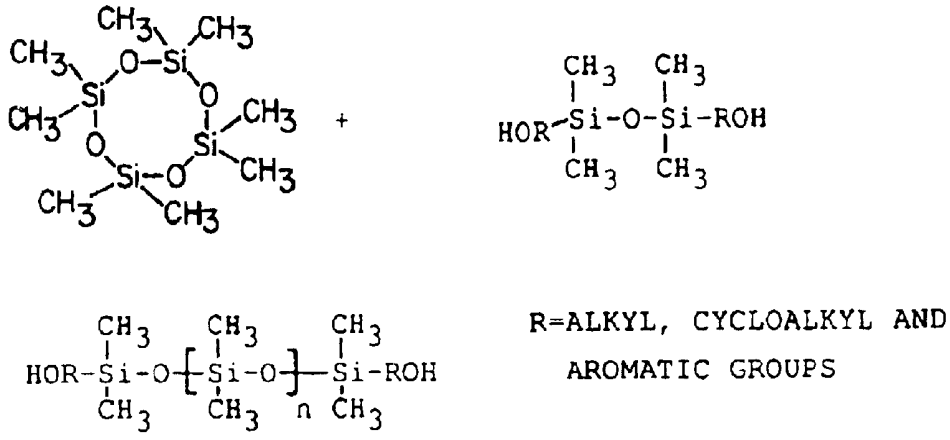


FIG. 7.

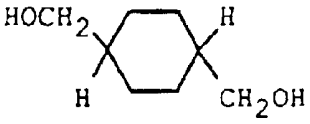
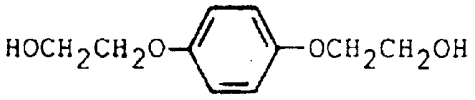
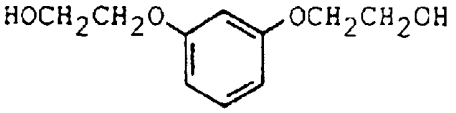
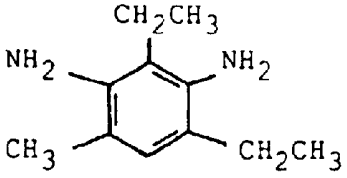
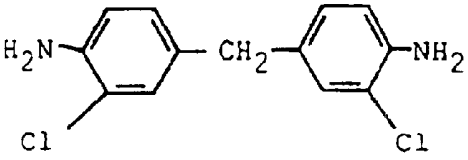
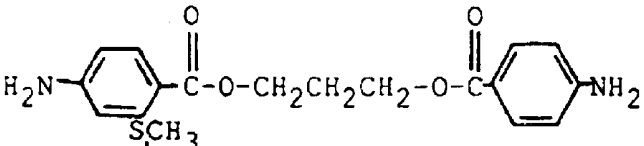
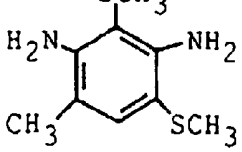
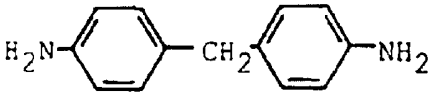
$HOCH_2CH_2OH$	ETHYLENE GLYCOL
$HOCH_2CH_2CH_2CH_2OH$	1,4-BUTANEDIOL
$HO(CH_2)_6OH$	1,6-HEXANEDIOL
	1,4-BIS(HYDROXYMETHYL) CYCLOHEXANE CIS AND TRANS
	P-DI(2-HYDROXYETHOXY)BENZENE HQEE
	M-DI(2-HYDROXYETHOXY)BENZENE HER
$H_2NCH_2CH_2NH_2$	ETHYLENEDIAMINE
	2,4-DIAMINO-3,5 DIETHYLTOLUENE ETHACURE 100 2 ISOMERS
	3,3'-DICHLORO-4,4'DIAMINODI-PHENYLMETHANE MOCA
	TRIMETHYLENE GLYCOL DI P-AMINO BENZOATE POLACURE 740 M
	2,4 DIAMINO 3,5 DI (METHLYTHIO) TOLUENE ETHACURE 300
	METHYLENEDIANILINE MDA

FIG. 8.

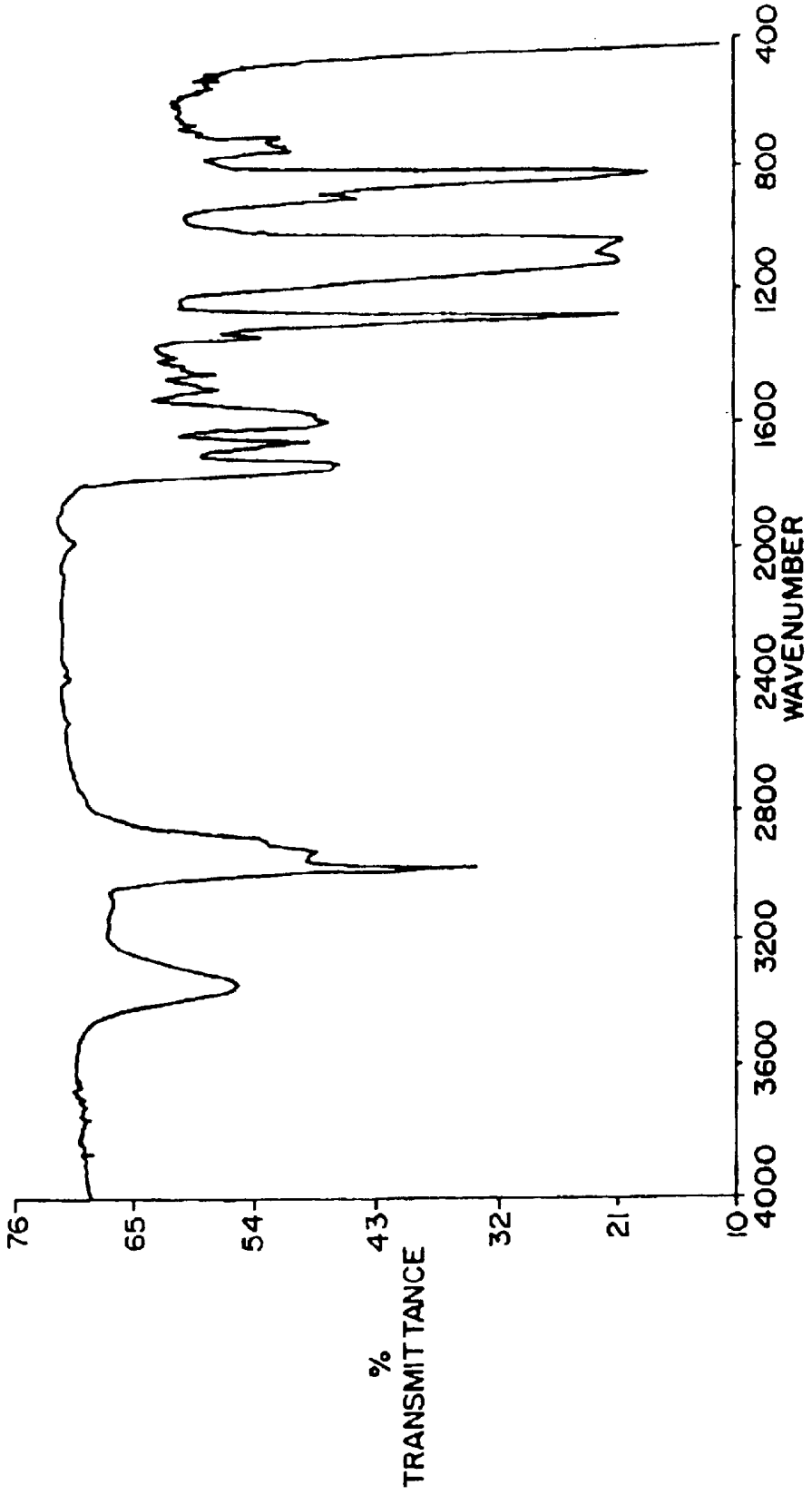

76
65
54
43
32
21
10
% TRANSMITTANCE
4000
3600
3200
2800
2400
2000
1600
1200
800
400
WAVENUMBER

FIG. 9.

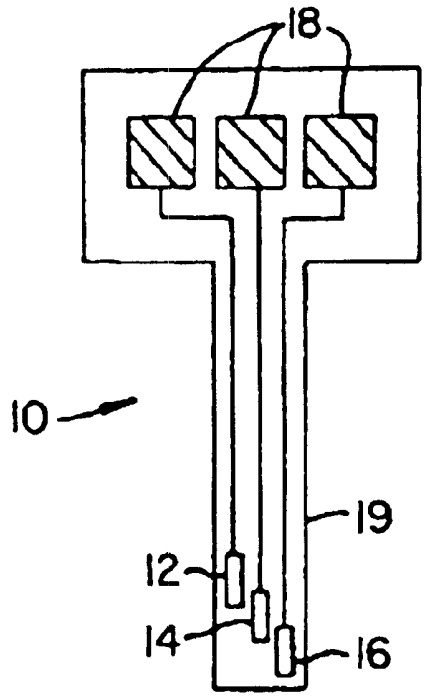


FIG. 10A.

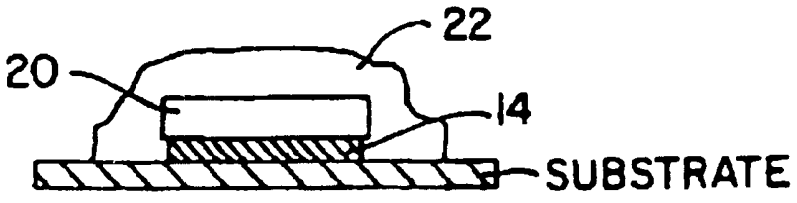


FIG. 10B.

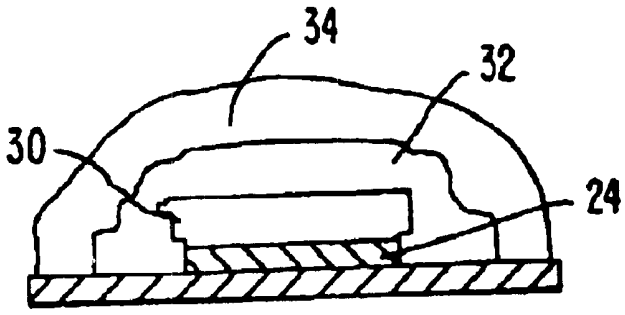


FIG. 10C.

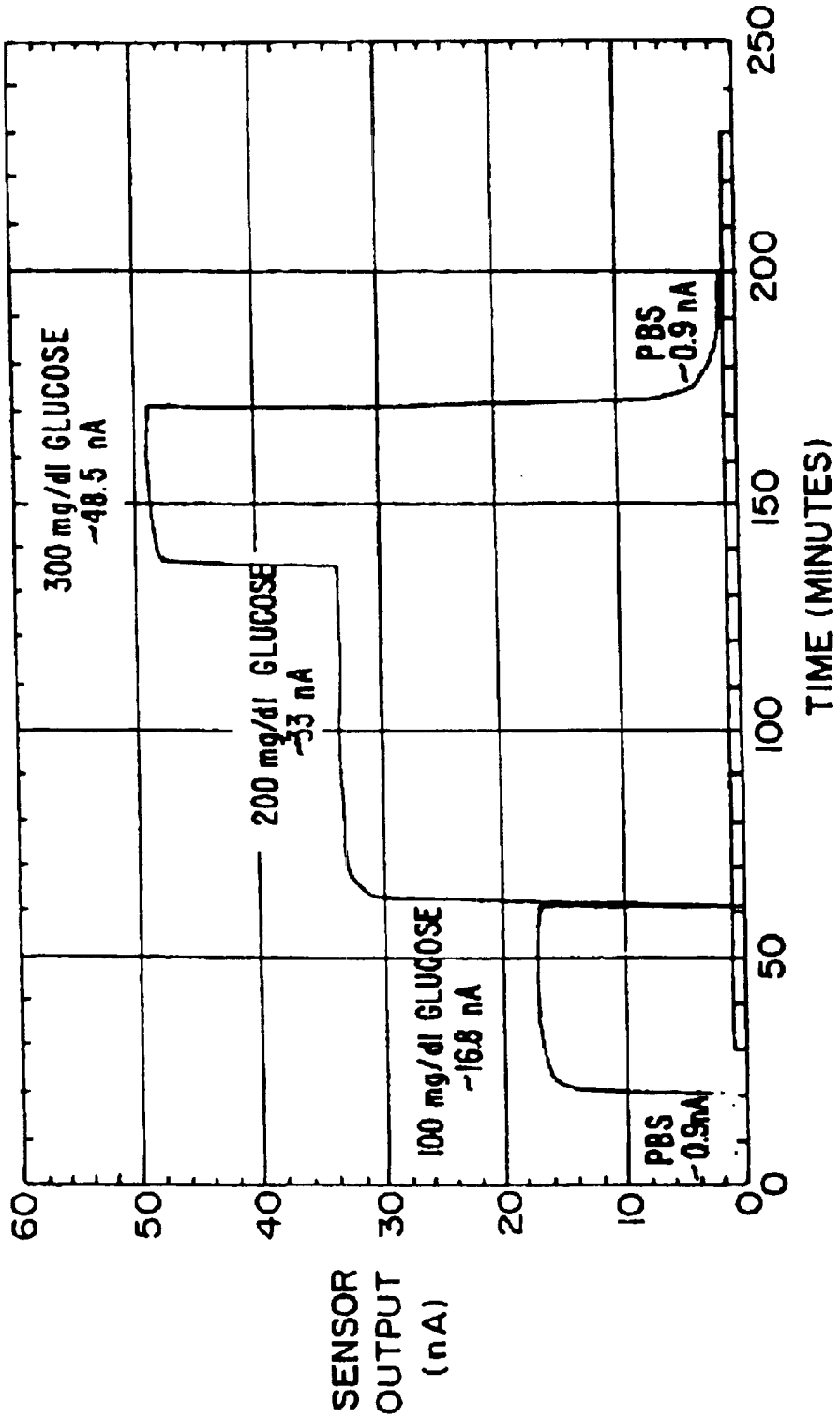

300 mg/dl GLUCOSE
~48.5 nA
200 mg/dl GLUCOSE
~33 nA
100 mg/dl GLUCOSE
~16.8 nA
PBS
~0.9 nA
PBS
~0.9nA
SENSOR OUTPUT (nA)
TIME (MINUTES)
0
10
20
30
40
50
60
0
50
100
150
200
250

FIG. 11.

HYDROPHILIC, SWELLABLE COATINGS FOR BIOSENSORS

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a continuation application of U.S. patent application Ser. No. 09/123,930, Jul. 28, 1998 now U.S. Pat. No. 6,462,162, which is a continuation application of U.S. patent application Ser. No. 08/749,754, Oct. 24, 1996 now U.S. Pat. No. 5,786,439; and this application is related to U.S. Ser. No. 08/721,262, now U.S. Pat. No. 5,770,060 which is a Continuation-in-Part of U.S. Ser. No. 08/410,775, which is now abandoned, the complete disclosures of each being incorporated herein by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention lies in the field of polymer chemistry in which the polymers formed are suitable for coating biosensors. The coatings act to decrease the impedance at the sensor's electrode and thereby enhance the signal during in vivo placement of the sensor.

2. Description of Related Art

Biosensors are small devices that use biological recognition properties for selective analysis of various analytes or biomolecules. Typically, the sensor will produce a signal that is quantitatively related to the concentration of the analyte. To achieve a quantitative signal, a recognition molecule or combination of molecules is often immobilized at a suitable transducer which converts the biological recognition event into a quantitative response.

A variety of biosensors have been developed for use with numerous analytes. Electroenzymatic biosensors use enzymes to convert a concentration of analyte to an electrical signal. Immunological biosensors rely on molecular recognition of an analyte by, for example, antibodies. Chemoreceptor biosensors use chemoreceptor arrays such as those of the olfactory system or nerve fibers from the antennules of the blue crab *Callinectes sapidus* to detect the presence of amino acids in concentrations as low as 10^{-9} M. For a review of some of the operating principles of biosensors, see Bergveld, et al., ADVANCES IN BIOSENSORS, Supplement 1, p. 31–91, Turner ed., and Collison, et al., Anal. Chem 62:425–437 (1990).

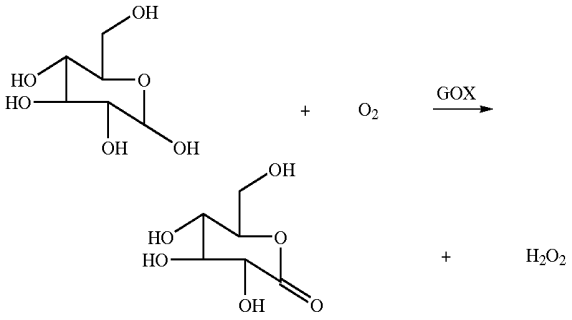
Regardless of the type of biosensor, each must possess certain properties to function in vivo and provide an adequate signal. First, the elements of the biosensor must be compatible with the tissue to which it is attached and be adequately shielded from adjacent tissues such that allergic or toxic effects are not exerted. Further, the sensor should be shielded from the environment to control drift in the generated signal. Finally, the sensor should accurately measure the analyte in the presence of proteins, electrolytes and medications which may interfere.

The prototype biosensor is the amperometric glucose sensor. There are several reasons for the wide ranging interest in glucose sensors. In the healthcare arena, glucose sensors are useful for glucose monitoring of patients with diabetes mellitus. Additionally, a working glucose sensor is required for the development of a closed loop artificial pancreas with an implanted insulin pump. A commercial interest focuses on sensors that can be used to monitor fermentation reactions in the biotechnology arena. From a scientific standpoint, interest is driven by the availability of a very robust enzyme, glucose oxidase, which can be used

to monitor glucose, as well as the desire to develop model sensors for a wide variety of analytes.

Any amperometric glucose sensor or any oxido-reductase enzyme that uses O_2 as a co-substrate and is designed for subcutaneous or intravenous use requires both an outer membrane and an anti-interference membrane. The requirement of two distinct membranes is due to the fundamental nature of the sensor as well as the environment in which the measurement is made.

A glucose sensor works according to the following chemical reaction (Equation 1):



In this reaction, glucose reacts with oxygen in the presence of glucose oxidase (GOX) to form gluconolactone and hydrogen peroxide. The gluconolactone further reacts with water to hydrolyze the lactone ring and produce gluconic acid. The H_2O_2 reacts electrochemically as shown below (Equation 2):

$$H_2O_2 \rightarrow O_2 + 2e^- 2H^+ \quad \text{(II)}$$

The current measured by the sensor/potentiostat (+0.5 to +0.7 v oxidation at Pt black electrode) is due to the two electrons generated by the oxidation of the H_2O_2. Alternatively, one can measure the decrease in the oxygen by amperometric measurement (−0.5 to −1 V reduction at a Pt black electrode).

The stoichiometry of Equation 1 clearly demonstrates some of the problems with an implantable glucose sensor. If there is excess oxygen for Equation 1, then the H_2O_2 is stoichiometrically related to the amount of glucose that reacts at the enzyme. In this case, the ultimate current is also proportional to the amount of glucose that reacts with the enzyme. If there is insufficient oxygen for all of the glucose to react with the enzyme, then the current will be proportional to the oxygen concentration, not the glucose concentration. For the sensor to be a true glucose sensor, glucose must be the limiting reagent, i.e. the O_2 concentration must be in excess for all potential glucose concentrations. For a number of conditions, this requirement is not easily achieved. For example, the glucose concentration in the body of a diabetic patient can vary from 2 to 30 mM (millimoles per liter or 36 to 540 mg/dl), whereas the typical oxygen concentration in the tissue is 0.02 to 0.2 mM (see, Fisher, et al., Biomed. Biochem. Acta. 48:965–971 (1989). This ratio in the body means that the sensor would be running in the Michaelis Menten limited regime and would be very insensitive to small changes in the glucose concentration. This problem has been called the "oxygen deficit problem". Accordingly, a method or system must be devised to either increase the O_2 in the GOX membrane, decrease the glucose concentration, or devise a sensor that does not use O_2.

Several approaches to solving the deficit problem have been attempted in the past. The simplest approach is to make

a membrane that is fully O_2 permeable, with no glucose permeability and mechanically perforate it with a small hole that allows glucose to pass. Here the differential permeability is defined by the ratio of the small hole area to the total membrane area. Two significant problems with this method are first that reproducibly making small holes is difficult and second and more serious, the O_2 permeability is a strong function of the thickness of the membrane and thickness is difficult to control in mass production. Microporous membranes (U.S. Pat. No. 4,759,828 to Young et al., incorporated herein by reference) have also been tried with limited success. Another problem with both the perforated membrane approach and the microporous membrane approach is that the sensor electrodes and the enzyme layer are exposed to body fluids. Body fluids contain proteins that coat the electrodes leading to decreased sensitivity of the sensor and enzymes (proteases) that can digest or degrade the sensor active enzyme.

Another approach to the oxygen deficit problem is described by Gough (U.S. Pat. No. 4,484,987, incorporated herein by reference). The approach uses a combination membrane with discrete domains of a hydrophilic material embedded in a hydrophobic membrane. In this case, the membrane is not homogenous and manufacturing reproducibility is difficult. Physical properties of the membrane are also compromised. In a similar manner, Gough (U.S. Pat. No. 4,890,620, incorporated herein by reference) describes a "two dimensional" system where glucose diffusion is limited to one dimension while the oxygen diffusion is from both dimensions. This sensor is extremely complicated and manufacturing on a large scale is expected to be difficult.

Several other groups have used a homogenous membrane of a relatively hydrophobic polyurethane and reported good results. See, for example, Shaw, et al., Biosensors and Bioelectronics, 6:401–406 (1991); Bindra, et al., Anal. Chem 63:1692 (1991); and Schichiri, et al., Horm. Metab. Resl. Suppl. Ser., 20:17 (1988). In classical diffusion experiments with these membranes, however, the glucose diffusion is extremely small. It is believed that the ability of these polyurethane layers to allow glucose diffusion is due to micro cracks or micro holes in these materials when applied as membranes.

Still others have developed homogeneous membranes with both hydrophilic and hydrophobic regions to circumvent the oxygen deficit problem. See, Allen et al., U.S. Pat. Nos. 5,284,140 and 5,322,063, the disclosures of each being incorporated herein by reference. These patents describe acrylic and polyurethane systems, respectively. Both of the membranes have hydrophilic and hydrophobic moieties in the molecule leading to limited control of oxygen and glucose permeabilities.

The key to stable, high sensitivity enzyme biosensors is that the sensor output must be limited only by the analyte of interest, not by any co-substrates or kinetically controlled parameters such as diffusion. In order to maximize the output current (Equation 2) of the biosensor, oxygen diffusion should be as large as possible while maintaining oxygen excess at the reaction surface. Since the normal concentration of O_2 in the subcutaneous tissue is quite low, maximization of the O_2 diffusion coefficient is desirable.

The membrane systems described in the literature as cited above attempt only to circumvent the oxygen deficit problem by reducing the amount of glucose diffusion to the working electrode of the biosensor. There is a need for the membrane to have physical stability and strength, adhesion to the substrate, processibility (ability to be synthesized/manufactured in reasonable quantities and at reasonable prices), biocompatibility, ability to be cut by laser ablation (or some other large scale processing method), and compatibility with the enzyme as deposited on the sensor.

Another one of the problems with implantable biosensors occurs as a result of "road block" type interference. This problem is encountered when the outermost layer of the biosensor has some hydrophobic characteristics. These characteristics result in the accumulation of plasma proteins on the surface of the electrode after only short periods of direct contact with body fluids. The hydrophobic regions of the sensor surface are believed to denature the proteins resulting in large deposits of protein mass. The deposits then affect the sensor's performance through a physical interference in a "road block" type of effect. The protein deposition is a gradual process which creates a non-uniform, non-predictable diffusion path for the analyte to the sensor. Moreover, the effect on the sensor is a cascading type in which the protein deposits dissipate the normal voltages applied to the electrodes (i.e., the deposits increase the capacitance of the system). The resultant requirement for higher voltages to offset the increased capacitance increases the noise, ultimately compromising the validity of the sensor's output.

Other problems are also associated with implantable sensors having hydrophobic regions at the sensor's surface. In particular, subcutaneous tissue contains substantial amounts of lipid vesicles. By implanting a biosensor directly into tissue, a portion of the sensor may be implanted directly into, or flush against a very hydrophobic lipid region. This also limits the aqueous environment which is required around the sensor's electrodes.

What is needed in the art are new coatings for implantable sensors which are extremely hydrophilic and provide a substantial and uniform aqueous flow around the sensors. Quite surprisingly, the present invention provides such coatings and sensors equipped with those coatings.

SUMMARY OF THE INVENTION

The present invention provides methods for reducing the electrode impedance of implantable biosensors by coating the surface of the biosensor with a uniform hydrogel which allows unimpeded water movement around the sensor. The surface coatings are compositions which are biocompatible and are capable of water uptake of at least 120% of their weight, more preferably at least 200% of their weight. Upon the uptake of water, the hydrogels used in the present invention will also swell and provide a layer of water around the electrodes to which the hydrogels are attached.

In one group of embodiments, the hydrogels can be prepared from (a) a diisocyanate, (b) a hydrophilic polymer which is a hydrophilic diol, a hydrophilic diamine, or a combination thereof, and optionally, (c) a chain extender.

The present invention also provides silicon containing compositions which are biocompatible and suitable for coating a biosensor. The compositions are polymers which are formed into membranes and can be prepared from: (a) a diisocyanate, (b) a hydrophilic polymer which is a hydrophilic diol, a hydrophilic diamine, or a combination thereof, (c) a siloxane polymer having functional groups at the chain termini, and optionally, (d) a chain extender.

The membranes prepared from the above components will have a glucose diffusion coefficient of from about 1×10^{-9} cm^2/sec to about 200×10^{-9} cm^2/sec, a water pickup of at least about 25% and a ratio of $D_{oxygen}/D_{glucose}$ of about 5 to about 200.

In certain preferred embodiments, the functional groups present in the siloxane polymer are amino, hydroxyl or

carboxylic acid, more preferably amino or hydroxyl groups. In other preferred embodiments, the hydrophilic polymer is a poly(ethylene)glycol which is PEG 200, PEG 400 or PEG 600. In still other preferred embodiments the diisocyanate is a isophorone diisocyanate, 1,6-hexamethylene diisocyanate or 4,4'-methylenebis(cyclohexyl isocyanate) and the chain extender is an alkylene diol, an alkylene diamine, an aminoalkanol or a combinations thereof.

In particularly preferred embodiments, the diisocyanate is 1,6-hexamethylene diisocyanate, the hydrophilic polymer is PEG 400 or PEG 600 and is present in an amount of about 17 to about 32 mol % (relative to all reactants), and the siloxane polymer is aminopropyl polysiloxane having a molecular weight of about 2000 to about 4000 and is present in an amount of about 17 to about 32 mol % (relative to all reactants).

The present invention further provides an implantable biosensor for measuring the reaction of an analyte, preferably glucose, and oxygen, the biosensor having a biocompatible membrane as described above. The present invention further provides implantable biosensors for measuring a variety of analytes, the biosensor having a coating as described above.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. **1**A and **1**B illustrate polymerization reactions of a diisocyanate with a poly(alkylene) glycol or a diamino poly(alkylene oxide) which results in a polyurethane or polyurea, respectively.

FIGS. **2** and **3** provide the structures of certain aliphatic and aromatic diisocyanates which are useful in forming the coatings described below.

FIG. **4** provides the structures of a number of hydrophilic polymers including poly(alkylene) glycols and diamino poly (alkylene oxides) which are used in polymers described below.

FIG. **5**A provides the structures of some chain extenders which are useful in the present compositions. This include aliphatic diols, diamines and alkanolamines and further include some aromatic diols and diamines. FIG. **5**B provides the structures of certain silicones which are useful in forming the membranes described below.

FIGS. **6** and **7** provides synthetic procedures for the preparation of some silicone polymers used in the present invention.

FIG. **8** provides the structures of some chain extenders which are useful in the present compositions. This include aliphatic diols, diamines and alkanolamines and further include some aromatic diols and diamines.

FIG. **9** is an infrared spectrum of a polyurea composition prepared in accordance with the present invention.

FIG. **10** illustrates portions of a glucose sensor which can be coated with a membrane of the present invention. FIG. **10**A is a schematic top view of a glucose sensor having electrodes covered with a polymer composition of the invention. FIG. **10**B is a sectional side view of a working electrode of the sensor which is covered with layers of an enzyme and a polymer composition of the invention. FIG. **10**C is a sectional side view of a working electrode of the sensor which is covered with layers of an enzyme, a glucose-limiting polymer and a hydrogel composition of the invention.

FIG. **11** is a graph showing sensor output in various glucose solutions as a function of time.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The following abbreviations are used herein: dl, deciliter, DEG, diethylene glycol; DMF, dimethyl formamide; PBS, phosphate buffered saline; THF, tetrahydrofuran; DI, deionized; PEG, poly(ethylene)glycol; HDI, 1,6-hexane diisocyanate (1,6hexamethylene diisocyanate); TMDI, 2,2,4,4-tetramethyl-1,6-hexane diisocyanate and 2,4,4-trimethyl-1,6-hexane diisocyanate; CHDI, 1,4-cyclohexane diisocyanate; BDI, 1,4cyclohexane bis(methylene isocyanate); H_6 XDI, 1,3-cyclohexane bis(methylene isocyanate) or hexahydro metaxylene diisocyanate; IPDI, isophorone diisocyanate; and H_{12} MDI, 4,4'dicyclohexylmethane diisocyanate; mv, millivolts.

As used herein, the term "polyurethane/polyurea" refers to a polymer containing urethane linkages, urea linkages or combinations thereof. Typically, such polymers are formed by combining diisocyanates with alcohols and/or amines. For example, combining isophorone diisocyanate with PEG 600 and 1,4-diaminobutane under polymerizing conditions provides a polyurethane/polyurea composition having both urethane (carbamate) linkages and urea linkages (see FIG. **1**A).

A. Hydrophilic Swellable Coatings for Biosensors

Methods for Reducing Electrode Impedance of Biosensors

In one aspect, the present invention provides methods for reducing electrode impedance of biosensors by coating the biosensor with an extremely hydrophilic polymer such as a hydrogel or a cellulose acetate. Typically, the polymer is applied to the surface of the sensor by spin coating, dipping or spraying. Methods of spraying including traditional methods as well as microdeposition techniques with an inkjet type of dispenser. Additionally, the polymer can be deposited on a sensor using photo-patterning to place the polymer on only specific portions of the sensor. This coating of the sensor provides a uniform water layer around the sensor which allows for improved diffusion of various analytes to the sensor.

A hydrogel is a highly-interdependent, biphasic matrix consisting of a solid component (usually a polymer, and more commonly a highly cross-linked polymer) that has both hydrophilic and hydrophobic character. Additionally, the matrix has a liquid component (e.g., water) that is retained in the matrix by intermolecular forces. The hydrophobic character provides the matrix with a degree of water insolubility while the hydrophilic character affords water permeability.

The polymer portion of the hydrogel will contain functionality which is suitable for hydrogen bonding (e.g., hydroxyl groups, amino groups, ether linkages, carboxylic acids and esters, and the like). Moreover, the affinity for water presented by the hydrogen bonding functionality must be of sufficient degree that the hydrated hydrogel will retain the water within its matrix even upon placement of the hydrogel in a hydrophobic medium such as an oil or lipid matrix. In addition to this binding of water within the hydrogel matrix, the hydrogel should allow water to flow through it when placed in an aqueous environment. A number of hydrogels have been developed for use as contact lenses. These hydrogels keep a layer of water at the surface of the eye to protect the eye from drying out.

The hydrogels used in coating the biosensors will typically be a polyurea, a polyurethane or a polyurethane/polyurea combination. FIG. **1**A illustrates some of the polymerization reactions which result in the compositions of the present invention.

Hydrogel Components

The hydrogels which are used in the present invention are prepared from the reaction of a diisocyanate and a hydrophilic polymer, and optionally, a chain extender. The hydrogels are extremely hydrophilic and will have a water pickup

of from about 120% to about 400% by weight, more preferably from about 150% to about 400%.

The diisocyanates which are useful in this aspect of the invention are those which are typically used in the preparation of biocompatible polyurethanes. Such diisocyanates are described in detail in Szycher, SEMINAR ON ADVANCES IN MEDICAL GRADE POLYURETHANES, Technomic Publishing, (1995) and include both aromatic and aliphatic diisocyanates (see FIGS. **2** and **3**). Examples of suitable aromatic diisocyanates include toluene diisocyanate, 4,4'-diphenylmethane diisocyanate, 3,3'-dimethyl-4,4'-biphenyl diisocyanate, naphthalene diisocyanate and paraphenylene diisocyanate. Suitable aliphatic diisocyanates include, for example, 1,6-hexamethylene diisocyanate (HDI), trimethyhexamethylene diisocyanate (TMDI), trans-1,4-cyclohexane diisocyanate (CHDI), 1,4-cyclohexane bis(methylene isocyanate) (BDI), 1,3-cyclohexane bis(methylene isocyanate) (H_6 XDI), isophorone diisocyanate (IPDI) and 4,4'-methylenebis(cyclohexyl isocyanate) (H_{12} MDI). In preferred embodiments, the diisocyanate is an aliphatic diisocyanate, more preferably isophorone diisocyanate, 1,6-hexamethylene diisocyanate, or 4,4'-methylenebis(cyclohexyl isocyanate). A number of these diisocyanates are available from commercial sources such as Aldrich Chemical Company Milwaukee, Wis., U.S.A.) or can be readily prepared by standard synthetic methods using literature procedures.

The quantity of diisocyanate used in the reaction mixture for the present compositions is typically about 50 mol % relative to the combination of the remaining reactants. More particularly, the quantity of diisocyanate employed in the preparation of the present compositions will be sufficient to provide at least about 100% of the —NCO groups necessary to react with the hydroxyl or amino groups of the remaining reactants. For example, a polymer which is prepared using x moles of diisocyanate, will use a moles of a hydrophilic polymer (diol, diamine or combination), and b moles of a chain extender, such that x=a+b, with the understanding that b can be zero.

A second reactant used in the preparation of the swellable coatings described herein is a hydrophilic polymer. The hydrophilic polymer can be a hydrophilic diol, a hydrophilic diamine or a combination thereof. The hydrophilic diol can be a poly(alkylene)glycol, a polyester-based polyol, or a polycarbonate polyol (see FIG. **4**). As used herein, the term "poly(alkylene)glycol" refers to polymers of lower alkylene glycols such as poly(ethylene)glycol, poly(propylene)glycol and polytetramethylene ether glycol (PnTEG). The term "polyester-based polyol" refers to a polymer as depicted in FIG. **4** in which the R group is a lower alkylene group such as ethylene, 1,3-propylene, 1,2-propylene, 1,4-butylene, 2,2-dimethyl-1,3-propylene, and the like. One of skill in the art will also understand that the diester portion of the polymer can also vary from the six-carbon diacid shown. For example, while FIG. **4** illustrates an adipic acid component, the present invention also contemplates the use of succinic acid esters, glutaric acid esters and the like. The term "polycarbonate polyol" refers those polymers having hydroxyl functionality at the chain termini and ether and carbonate functionality within the polymer chain (see FIG. **4**). The alkyl portion of the polymer will typically be composed of C2 to C4 aliphatic radicals, or in some embodiments, longer chain aliphatic radicals, cycloaliphatic radicals or aromatic radicals. The term "hydrophilic diamines" refers to any of the above hydrophilic diols in which the terminal hydroxyl groups have been replaced by reactive amine groups or in which the terminal hydroxyl groups have been derivatized to produce an extended chain having terminal amine groups. For example, a preferred hydrophilic diamine is a "diamino poly(oxyalkylene)" which is poly(alkylene)glycol in which the terminal hydroxyl groups are replaced with amino groups. The term "diamino poly(oxyalkylene" also refers to poly(alkylene) glycols which have aminoalkyl ether groups at the chain termini. One example of a suitable diamino poly (oxyalkylene) is poly(propylene glycol) bis(2-aminopropyl ether). A number of diamino poly(oxyalkylenes) are available having different average molecular weights and are sold as Jeffamines™ (for example, Jeffamine 230, Jeffamine 600, Jeffamine 900 and Jeffamine 2000). These polymers can be obtained from Aldrich Chemical Company. Alternatively, literature methods can be employed for their synthesis.

The amount of hydrophilic polymer which is used in the present compositions will typically be about 10% to about 100% by mole relative to the diisocyanate which is used. Preferably, the amount is from about 50% to about 90% by mole relative to the diisocyanate. When amounts less than 100% of hydrophilic polymer are used, the remaining percentage (up to 100%) will be a chain extender.

Thus, in one group of embodiments, the reaction mixture for the preparation of swellable coatings will also contain a chain extender which is an aliphatic or aromatic diol, an aliphatic or aromatic diamine, alkanolamine, or combinations thereof. Examples of suitable aliphatic chain extenders include ethylene glycol, propylene glycol, 1,4-butanediol, 1,6-hexanediol, ethanolamine, ethylene diamine, butane diamine and 1,4-cyclohexanedimethanol. Aromatic chain extenders include, for example, para-di(2-hydroxyethoxy) benzene, meta-di(2-hydroxyethoxy)benzene, Ethacure 100™ (a mixture of two isomers of 2,4-diamino-3,5-diethyltoluene), Ethacure 300™ (2,4-diamino-3,5-di (methylthio)toluene), 3,3'-dichloro-4,4'-diaminodiphenylmethane, Polacure™ 740 M (trimethylene glycol bis(para-aminobenzoate)ester), and methylenedianiline. Incorporation of one or more of the above chain extenders typically provides the resulting biocompatible membrane with additional physical strength, but does not substantially alter the hydrophilicity of the polymer. In particularly preferred compositions, the chain extender is butanediol, ethylenediamine, 1,6-hexamethylenediamine, 1,2-diaminocyclohexane or isophorone diamine. In one group of preferred embodiments, the chain extender is present an amount of from about 10% to 50% by mole relative to the diisocyanate.

Coating Preparation

Polymerization of the above reactants can be carried out in bulk or in a solvent system. Use of a catalyst is preferred, though not required. Suitable catalysts include dibutyltin bis(2-ethylhexanoate), dibutyltin diacetate, triethylamine and combinations thereof. Preferably dibutyltin bis(2-ethylhexanoate is used as the catalyst. Bulk polymerization is typically carried out at an initial temperature of about 25° C. (ambient temperature) to about 50° C., in order to insure adequate mixing of the reactants. Upon mixing of the reactants, an exotherm is typically observed, with the temperature rising to about 90° C.–120° C. After the initial exotherm, the reaction flask can be heated at from 75° C. to 125° C., with 90° C. to 100° C. being a preferred temperature range. Heating is usually carried out for one to two hours.

Solution polymerization can be carried out in a similar manner. Solvents which are suitable for solution polymerization include, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, dimethylacetamide, halogenated solvents

such as 1,2,3-trichloropropane, and ketones such as 4-methyl-2-pentanone. Preferably, THF is used as the solvent. When polymerization is carried out in a solvent, heating of the reaction mixture is typically carried out for at least three to four hours, and preferably at least 10–20 hours. At the end of this time period, the solution polymer is typically cooled to room temperature and poured into DI water. The precipitated polymer is collected, dried, washed with hot DI water to remove solvent and unreacted monomers, then re-dried. The dried polymer can be evaluated for water pickup as described in the Examples below.

The hydrogels which are useful in the present invention will have a water pickup of at least 120%, preferably 150% to about 400%, and more preferably about 200% to about 400%.

Polymers prepared by bulk polymerization are typically dissolved in dimethylformamide and precipitated from water. Polymers prepared in solvents such as THF can be poured into water at ambient temperatures, then filtered, dried, washed with boiling water and re-dried.

Once the polymers have been prepared having suitable water pickup, the polymers can be solubilized in a solvent and used to coat a biosensor.

Preparation of coated biosensors can be accomplished by dissolving the dried polymer in a suitable solvent and spin-coating the sensor, typically using, for example, a 5 wt % in 2-propanol solution of the polymer. The selection of other suitable solvents for coating the sensors will typically depend on the particular polymer as well as the volatility of the solvent. Other suitable solvents include THF, $CHCl_3$, CH_2Cl_{12}, DMF or combinations thereof. More preferably, the solvent is THF or DMF/CH_2Cl_2 (2/98 volume %).

A number of different sensors can be used in the methods and compositions of the present invention.

B. Silicon-Containing Biocompatible Membranes

Biocompatible Membranes

As noted herein, requirements for a glucose sensor intended for in vivo use is that the supply of oxygen in the vicinity of the sensing element not be depleted. Additionally, the glucose should diffuse to the sensor at a controlled rate. This does not mean that a glucose sensor membrane need have an extremely high permeability to oxygen. Instead, the membrane should control the relative rates of diffusion of oxygen and glucose to the sensor so that the local concentration of oxygen is not depleted. Additionally, the glucose sensors intended for in vivo use must also be biocompatible with the body, and they must be able to function in an environment in which acids are present as well as proteins which can interfere with a sensor. Thus, the enzyme(s) used in such sensors must be protected from degradation or denaturation, while the elements of such sensors must be protected from molecules which would foul the sensors or their accuracy will decrease over time.

In one aspect, the present invention provides a biocompatible membrane formed from a reaction mixture of:

(a) a diisocyanate, said diisocyanate comprising about 50 mol % of the reactants in said mixture;

(b) a hydrophilic polymer which is a member selected from the group consisting of a hydrophilic diol, a hydrophilic diamine and combinations thereof; and

(c) a silicone polymer having functional groups at the chain termini. Optionally, the reaction mixture will contain a chain extender. The membrane formed using the polymerized mixture of the above components will have a glucose diffusion coefficient of from about 1 to about 200×10^{-9} cm^2/sec, a water pickup of at least 25% and a ratio of $D_{oxygen/Dglucose}$ of from about 5 to about 200.

Depending on the selection of components, the polymer used in forming the biocompatible membranes will be a polyurea, a polyurethane or a polyurethane/polyurea combination. FIG. **1B** illustrates some of the polymerization reactions which result in the compositions of the present invention.

Membrane Components

The homogeneous membranes of the invention are prepared from biologically acceptable polymers whose hydrophobic/hydrophilic balance can be varied over a wide range to control the ratio of the diffusion coefficient of oxygen to that of glucose, and to match this ratio to the design requirements of electrochemical glucose sensors intended for in vivo use. Such membranes can be prepared by conventional methods by the polymerization of monomers and polymers noted above. The resulting polymers are soluble in solvents such as acetone or ethanol and may be formed as a membrane from solution by dip, spray or spin coating.

The diisocyanates which are useful in this aspect of the invention are those which are typically those which are used in the preparation of biocompatible polyurethanes. Such diisocyanates are described in detail in Szycher, SEMINAR ON ADVANCES IN MEDICAL GRADE POLYURETHANES, Technomic Publishing, (1995) and include both aromatic and aliphatic diisocyanates (see FIGS. **2** and **3**). Examples of suitable aromatic diisocyanates include toluene diisocyanate, 4,4'-diphenylmethane diisocyanate, 3,3'-dimethyl-4,4'-biphenyl diisocyanate, naphthalene diisocyanate and paraphenylene diisocyanate. Suitable aliphatic diisocyanates include, for example, 1,6hexamethylene diisocyanate (HDI), trimethylhexamethylene diisocyanate II), trans1,4-cyclohexane diisocyanate (CHDI), 1,4-cyclohexane bis(methylene isocyanate) (BDI), 1,3-cyclohexane bis(methylene isocyanate) (H_6 XDI), isophorone diisocyanate (IPDI) and 4,4'-methylenebis (cyclohexyl isocyanate) (H_2 MDI). In preferred embodiments, the diisocyanate is isophorone diisocyanate, 1,6-hexamethylene diisocyanate, or 4,4'-methylenebis (cyclohexyl isocyanate). A number of these diisocyanates are available from commercial sources such as Aldrich Chemical Company (Milwaukee, Wis., U.S.A.) or can be readily prepared by standard synthetic methods using literature procedures.

The quantity of diisocyanate used in the reaction mixture for the present compositions is typically about 50 mol % relative to the combination of the remaining reactants. More particularly, the quantity of diisocyanate employed in the preparation of the present compositions will be sufficient to provide at least about 100% of the —NCO groups necessary to react with the hydroxyl or amino groups of the remaining reactants. For example, a polymer which is prepared using x moles of diisocyanate, will use a moles of a hydrophilic polymer (diol, diamine or combination), b moles of a silicone polymer having functionalized termini, and c moles of a chain extender, such that x=a+b+c, with the understanding that c can be zero.

A second reactant used in the preparation of the biocompatible membranes described herein is a hydrophilic polymer. The hydrophilic polymer can be a hydrophilic diol, a hydrophilic diamine or a combination thereof. The hydrophilic diol can be a poly(alkylene)glycol, a polyester-based polyol, or a polycarbonate polyol (see FIG. **4**). As used herein, the term "poly(alkylene)glycol" refers to polymers of lower alkylene glycols such as poly(ethylene)glycol, poly(propylene)glycol and polytetramethylene ether glycol (PTMEG). The term "polyester-based polyol" refers to a

polymer as depicted in FIG. **4** in which the R group is a lower alkylene group such as ethylene, 1,3-propylene, 1,2-propylene, 1,4-butylene, 2,2-dimethyl-1,3-propylene, and the like. One of skill in the art will also understand that the diester portion of the polymer can also vary from the six-carbon diacid shown. For example, while FIG. **4** illustrates an adipic acid component, the present invention also contemplates the use of succinic acid esters, glutaric acid esters and the like. The term "polycarbonate polyol" refers those polymers having hydroxyl functionality at the chain termini and ether and carbonate functionality within the polymer chain (see FIG. **4**). The alkyl portion of the polymer will typically be composed of C2 to C4 aliphatic radicals, or in some embodiments, longer chain aliphatic radicals, cycloaliphatic radicals or aromatic radicals. The term "hydrophilic diamines" refers to any of the above hydrophilic diols in which the terminal hydroxyl groups have been replaced by reactive amine groups or in which the terminal hydroxyl groups have been derivatized to produce an extended chain having terminal amine groups. For example, a preferred hydrophilic diamine is a "diamino poly (oxyalkylene)" which is poly(alkylene)glycol in which the terminal hydroxyl groups are replaced with amino groups. The term "diamino poly(oxyalkylene" also refers to poly (alkylene)glycols which have aminoalkyl ether groups at the chain termini. One example of a suitable diamino poly (oxyalkylene) is poly(propylene glycol)bis(2-aminopropyl ether). A number of the above polymers can be obtained from Aldrich Chemical Company. Alternatively, literature methods can be employed for their synthesis.

The amount of hydrophilic polymer which is used in the present compositions will typically be about 10% to about 80% by mole relative to the diisocyanate which is used. Preferably, the amount is from about 20% to about 60% by mole relative to the diisocyanate. When lower amounts of hydrophilic polymer are used, it is preferable to include a chain extender (see below).

Silicone polymers which are useful in the present invention are typically linear, have excellent oxygen permeability and essentially no glucose permeability. Preferably, the silicone polymer is a polydimethylsiloxane having two reactive functional groups (i.e., a functionality of 2). The functional groups can be, for example, hydroxyl groups, amino groups or carboxylic acid groups, but are preferably hydroxyl or amino groups (see FIG. **5**B). In some embodiments, combinations of silicone polymers can be used in which a first portion comprises hydroxyl groups and a second portion comprises amino groups. Preferably, the functional groups are positioned at the chain termini of the silicone polymer. A number of suitable silicone polymers are commercially available from such sources as Dow Chemical Company (Midland, Mich., U.S.A.) and General Electric Company (Silicones Division, Schenectady, N.Y., U.S.A.). Still others can be prepared by general synthetic methods as illustrated in FIGS. **6** and **7**, beginning with commercially available siloxanes (United Chemical Technologies, Bristol. Pa., U.S.A.). For use in the present invention, the silicone polymers will preferably be those having a molecular weight of from about 400 to about 10,000, more preferably those having a molecular weight of from about 2000 to about 4000. The amount of silicone polymer which is incorporated into the reaction mixture will depend on the desired characteristics of the resulting polymer from which the biocompatible membrane are formed. For those compositions in which a lower glucose penetration is desired, a larger amount of silicone polymer can be employed. Alternatively, for compositions in which a higher glucose penetration is desired, smaller amounts of silicone polymer can be employed. Typically, for a glucose sensor, the amount of siloxane polymer will be from 10% to 90% by mole relative to the diisocyanate. Preferably, the amount is from about 20% to 60% by mole relative to the diisocyanate.

In one group of embodiments, the reaction mixture for the preparation of biocompatible membranes will also contain a chain extender which is an aliphatic or aromatic diol, an aliphatic or aromatic diamine, alkanolamine, or combinations thereof (see FIG. **8**). Examples of suitable aliphatic chain extenders include ethylene glycol, propylene glycol, 1,4-butanediol, 1,6-hexanediol, ethanolamine, ethylene diamine, butane diamine, 1,4-cyclohexanedimethanol. Aromatic chain extenders include, for example, para-di(2-hydroxyethoxy)benzene, meta-di(2-hydroxyethoxy) benzene, Ethacure 1001™ (a mixture of two isomers of 2,4-diamino-3,5-diethyltoluene), Ethacure 300™ (2,4-diamino-3,5-di(methylthio)toluene), 3,3'-dichloro-4, 4'diaminodiphenylmethane, Polacure™ 740M (trimethylene glycol bis(para-aminobenzoate)ester), and methylenedianiline. Incorporation of one or more of the above chain extenders typically provides the resulting biocompatible membrane with additional physical strength, but does not substantially increase the glucose permeability of the polymer. Preferably, a chain extender is used when lower (i.e., 10–40 mol %) amounts of hydrophilic polymers are used. In particularly preferred compositions, the chain extender is diethylene glycol which is present in from about 40% to 60% by mole relative to the diisocyanate.

Membrane Preparation

Polymerization of the above reactants can be carried out in bulk or in a solvent system. Use of a catalyst is preferred, though not required. Suitable catalysts include dibutyltin bis(2-ethylhexanoate), dibutyltin diacetate, triethylamine and combinations thereof. Preferably dibutyltin bis(2-ethylhexanoate is used as the catalyst. Bulk polymerization is typically carried out at an initial temperature of about 25° C. (ambient temperature) to about 50° C., in order to insure adequate nixing of the reactants. Upon mixing of the reactants, an exotherm is typically observed, with the temperature rising to about 90° C.–120° C. After the initial exotherm, the reaction flask can be heated at from 75° C. to 125° C., with 90° C. to 100° C. being a preferred temperature range. Heating is usually carried out for one to two hours. Solution polymerization can be carried out in a similar manner. Solvents which are suitable for solution polymerization include dimethylformamide, dimethyl sulfoxide, dimethylacetamide, halogenated solvents such as 1,2,3-trichloropropane, and ketones such as 4-methyl-2-pentanone. Preferably, THF is used as the solvent. When polymerization is carried out in a solvent, heating of the reaction mixture is typically carried out for three to four hours.

Polymers prepared by bulk polymerization are typically dissolved in dimethylformamide and precipitated from water. Polymers prepared in solvents that are not miscible with water can be isolated by vacuum stripping of the solvent. These polymers are then dissolved in dimethylformamide and precipitated from water. After thoroughly washing with water, the polymers can be dried in vacuo at about 50° C. to constant weight.

Preparation of the membranes can be completed by dissolving the dried polymer in a suitable solvent and cast a film onto a glass plate. The selection of a suitable solvent for casting will typically depend on the particular polymer as well as the volatility of the solvent. Preferably, the solvent is THF, $CHCl_3$, CH_2Cl_2, DMF or combinations thereof.

More preferably, the solvent is THF or DMF/CH_2Cl_2 (2/98 volume %), the solvent is removed from the films, the resulting membranes are hydrated fully, their thicknesses measured and water pickup is determined. Membranes which are useful in the present invention will typically have a water pickup of about 20 to about 100%, preferably 30 to about 90%, and more preferably 40 to about 80%, by weight.

Oxygen and glucose diffusion coefficients can also be determined for the membranes of the present invention. Methods for determining diffusion coefficients are known to those of skill in the art, and examples are provided below. The biocompatible membranes described herein will preferably have a oxygen diffusion coefficient (D_{oxygen}) of about 0.1×10^{-6} cm^2/sec to about 2.0×10^{-6} cm^2/sec and a glucose diffusion coefficient ($D_{glucose}$) of about 1×10^{-9} cm^2/sec to about 500×10^{-9} cm^2/sec. More preferably, the glucose diffusion coefficient is about 10×10^{-9} cm^2/sec to about 200×10^{-9} cm^2/sec.

From the above description, it will be apparent to one of skill in the art that the discovery underlying the present invention is the use of silicon-containing polymers, such as siloxanes, in the formation of biocompatible membranes. The silicon-containing polymers are used in conjunction with (covalently attached to) hydrophilic polymers for the preparation of membranes in which the movement of analytes and reactive species (e.g., oxygen and glucose) can be controlled by varying the amounts of each component. The membranes produced from these components are homogeneous and are useful for coating a number of biosensors and devices designed for subcutaneous implantation.

C. Membrane-Coated Biosensors

Glucose sensors which utilize, for example, glucose oxidase to effect a reaction of glucose and oxygen are known in the art, and are within the skill in the art to fabricate. See, for example, U.S. Pat. Nos. 5,165,407, 4,890,620, 5,390,671 and 5,391,250, the disclosures of each being incorporated herein by reference. The present invention depends not on the configuration of the biosensor, but rather on the use of the inventive membranes to cover or encapsulate the sensor elements.

In particular, the hydrogels described herein are particularly useful with a variety of biosensors for which it is advantageous to provide a surrounding water layer for the electrodes. In addition, the biocompatible membranes of the present invention are useful with a variety of biosensors for which it is advantageous to control diffusion of the analytes/reactants to the sensing elements. Various such biosensors are well known in the art. For example, sensors for monitoring glucose concentration of diabetics are described in Shichiri, et al.,: "In Vivo Characteristics of Needle-Type Glucose Sensor-Measurements of Subcutaneous Glucose Concentrations in Human Volunteers," Horm. Metab. Res., Suppl. Ser. 20:17–20 (1988); Bruckel, et al.,: "In Vivo Measurement of Subcutaneous Glucose Concentrations with an Enzymatic Glucose Sensor and a Wick Method," Klin. Wochenschr. 67:491–495 (1989); and Pickup, et al.,: "In Vivo Molecular Sensing in Diabetes Mellitus: An Implantable Glucose Sensor with Direct Electron Transfer," Diabetologia 32:213–217 (1989).

Other sensors are described in, for example Reach, et al., in ADVANCES IN BIOSENSORS, A. Turner (ed.), JAI Press, London, Chap. 1, (1993), incorporated herein by reference.

The following examples are offered by way of illustration and are not meant to limit the scope of the invention.

EXAMPLES

The materials used in the examples were obtained from the following sources: isophorone diisocyanate, 1,6-hexamethylenediisocyanate, PEG 600, butanediol, ethylene diamine, hexamethylenediamine, isophorone diamine and 1,2-diaminohexane (Aldrich Chemical Co., Milwaukee, Wis., U.S.A.); Jeffamine™ D-230, ED-600, ED-900 and D-2000 were obtained from Aldrich.

Typical Examples of Hydrophilic Swellable Coatings for Biosensors

Examples 1–2

General Methods

(a) Hydrogel Preparation

Hydrogels suitable for use as biosensor coatings were prepared by combining a diisocyanate with an equivalent molar amount of a hydrophilic diol or diamine or with a combination of diol or diamine and chain extender such that the molar amount of the combination was equivalent to the diisocyanate. The polymerizations were carried out in a one-pot reaction using THF as solvent and a trace catalyst (tributyltin ethylhexanoate). The reactions were heated to reflux and held at this temperature overnight (about 16 hours). The resulting polymer solution was poured into a large volume of DI water at about 20° C. and then filtered, dried, and washed with boiling DI water. The resulting polymer was again dried then taken up in 2-propanol (as a 5 wt % solution) and used for spin coating.

(b) Coating of Biosensors

Coating of biosensors can be carried out using a commercial spin coating apparatus operating at between 1000 and 5000 rpm, depending on the viscosity of the polymer solution and the desired thickness of the hydrophilic coating.

(c) Water Pickup

Water pickup was determined gravimetrically at room temperature on polymers which had been dried to a constant weight at 50° C. in vacuo, then weighed, immersed in deionized water for 24 hours, removed and blotted with filter paper, and weighed. Percent water pickup was determined from the formula:

$$\%\ \text{Pickup}=(W_w-W_d)/W_d\times100$$

where W_w is the weight of the swollen film and W_d is the weight of the dry film.

(d) Impedance Measurements

Electrochemical impedance measurements were performed on finished sensors using a Bioanalytical Systems (BAS, Lafayette, Ind.) 100B Electrochemical Analyzer. Impedance was measured in a three electrode mode from 0.01 H to 1000 Hz. Linear extrapolation to DC impedance was used to obtain the final impedance figures. The final impedance is calculated as the sum of the real and imaginary parts of the impedance. The measurements were made in 100 mg/dl glucose solution in PBS, with a 600 mv applied potential and a 5 mv A.C. signal imposed on the applied potential.

Example 1

This example provides the formulations and properties of representative coatings. Table 1 provides ten formulations for representative polymers which were prepared by solution polymerization.

TABLE 1

Representative Polymer Formulations

Polymer	Diisocyanate	Hydrophilic diol or diamine	Chain Extender
1	1,6-Hexamethylene	Jeffamine 600 (95%)	Butanediol (5%)
2	1,6-Hexamethylene	Jeffamine 2000 (100%)	None
3	1,6-Hexamethylene	Jeffamine 2000 (90%)	Butanediol (10%)
4	1,6-Hexamethylene	PEG 2000 (90%)	Butanediol (10%)
5	1,6-Hexamethylene	Jeffamine 230 (30%)	Ethylene diamine (70%)
6	1,6-Hexamethylene	PEG 600 (75%)	Ethylene diamine (25%)
7	Isophorone	PEG 600 (75%)	Butanediol (25%)
8	Isophorone	Jeffamine 900 (70%)	1,6-Diaminohexane (25%)
9	Isophorone	Jeffamine 900 (50%)	1,2-Diaminocyclo-hexane (50%)
10	Isophorone	Jeffamine 900 (50%)	Isophorone diamine (50%)

Table 2 provides certain physical and chemical properties of the polymers above.

TABLE 2

Physical Properties of Representative Polymers

Polymer	Water Pickup (%)	Impedance (Ohms) ($x10^6$)
1	250	2.3
2	160	1.7
3	240	1.4
4	400	6.1
5	110	3.3
6	45	6.9
7	280	1.1
8	240	0.7
9	220	0.5
10	184	0.8

Example 2

This example illustrates the evaluation of a membrane-coated biosensor constructed present invention.

A membrane prepared from the polymer identified as 9 above was found to have excellent mechanical properties as well as appropriate water uptake and oxygen and glucose diffusivities. The membrane was evaluated using a prototype glucose sensor illustrated in FIG. **10**A According to a sensor **10** was constructed having a reference electrode **12**, a working electrode **14**, and a counter electrode **16** deposited on a polymeric sheet **19**. A series of bonding pads **18** complete the sensor **10**. As shown in FIG. **10**C, the working electrode **24** was covered with a layer **30** of the enzyme glucose oxidase and the entire electrode array was coated with a first layer **32** of a glucose-limiting polymer prepared according to U.S. Ser. No. 08/721,262 (filed Sep. 26, 1996 and incorporated herein by reference) and a second layer **34** of the polymer 9 (see Example 1) by spin coating. The glucose limiting polymer was applied from a 7 wt % solution of the polymer in THF and the hydrophilic coating **34** was applied from a 5 wt % solution in 2-propanol. The sensor was connected to a commercial potentiostat (BAS Instruments, not shown) and operated with a potential of +0.6 volts between the working electrode and the reference electrode.

Typical Examples of Silicon-Containing Biocompatible Membranes

Examples 3–6

The materials used in the following examples were obtained from commercial sources.

General Methods

(a) Membrane Preparation

Membranes were prepared by casting films from a suitable solvent onto glass plates using a parallel arm Gardner knife (Gardner Labs). The solvent chosen will depend on the particular chemical structure of the polymer. Typically, THF or DMF/CH_2Cl_2 (2/98 vol %) are used although chloroform is also useful as it is readily volatile. After removal of the solvent, the dried membranes were hydrated with deionized water for 30–60 minutes. The membranes were then removed and transferred to a Mylar™ support sheet. Wet film thicknesses were measured with a micrometer before removal from the support. Films were also cast from solution onto filtration membranes of known thickness. For the measurements provided below, it was assumed that the membrane material completely filled the pores of the filtration membranes and that the thickness of the filtration media is the thickness of the membrane.

(b) Diffusion Constants

Diffusion constants were measured in a standard permeability cell (Crown Glass Co., Inc.) maintained at 37° C., using Fick's relationship:

$$J=D\ dC/dx$$

where J is total flux, D is the diffusion constant of the analyte of interest, and dC/dx is the concentration gradient across the membrane. The diffusion coefficient is a physical property of both the analyte of interest and the material in which it is diffusing. Thus, D is a property of the system under evaluation.

Oxygen diffusion constants (D_o) were determined by securing the membrane with two rubber gaskets between the two halves of a diffusion cell maintained at 37° C., and clamping the two halves together. Each side of the cell was filled with phosphate buffered saline (PBS, 0.15M NaCl, 0.05M phosphate, pH 7.4). One side was saturated with HPLC grade helium while the other side was saturated with room air (assumed 20% O_2). A calibrated oxygen electrode Microelectrodes, Inc.) was placed in each cell. The oxygen electrode outputs were connected to a microcomputer-controlled data acquisition system and the oxygen concentration from both cells was recorded as a function of time. The curves of concentration vs. time were plotted and the diffusion coefficients were calculated using the entire curve. Curve fits generally had correlation coefficients (R^2) of greater than 0.95.

Glucose diffusion constants (D_G) were determined as above except that one half of the cell was filled with phosphate buffered saline containing 400 mg/dl of glucose. The concentration of glucose in each half of the cell was measured at 5 minute intervals until equilibrium was achieved using a YSI glucose analyzer. As above, the curves of concentration vs. time were plotted and the diffusion coefficient was calculated.

(c) Water Pickup

Water pickup was determined gravimetrically at room temperature on films which were less than 0.5 mm thick After evaporation of the casting solvent, films were dried to constant weight at 50° C. in vacuo, weighed, immersed in deionized water for 24 hours, removed and blotted with filter

paper, and weighed. Percent water pickup was determined from the formula:

$$\% \text{ Pickup} = (W_w - W_d)/W_d \times 100$$

where W_w is the weight of the swollen film and W_d is the weight of the dry film.

Example 3

This example illustrates a bulk polymerization method of polymer formation carried out with isophorone diisocyanate, PEG 600, diethylene glycol and aminopropyl terminated polydimethyl siloxane.

Isophorone diisocyanate (4.44 g, 20 mmol, 100 mol %) was dried over molecular sieves and transferred to a 100 mL round bottom flask fitted with a nitrogen purge line and a reflux condenser. PEG 600 (2.40 g, 4.0 mmol, 20 mol %), diethylene glycol (1.06 g, 10 mmol, 50 mol %) and aminopropyl terminated polydimethylsiloxane (15 g, 6.0 mmol, 30 mol %, based on a 2500 average molecular weight) were added to the flask Heating was initiated using a heating mantle until a temperature of 50° C. was obtained. Dibutyltin bis(2-ethylhexanoate) (15 mg) was added and the temperature increased to about 95° C. The solution was continuously stirred at a temperature of 65° C. for a period of 4 hr during which time the mixture became increasingly viscous. The resulting polymer was dissolved in 50 mL of hot THF and cooled. After cooling, the solution was poured into 5 L of stirring DI water. The precipitated polymer was torn into small pieces and dried at 50° C. until a constant weight was achieved.

Example 4

This example illustrates a solution polymerization method using 1,6-hexamethylene diisocyanate, PEG 200 and aminopropyl terminated polydimethylsiloxane.

Dried 1,6-hexamethylene diisocyanate (1.34 g, 8 mmol, 100 mol %) was added to a 100 mL 3-neck flask containing 20 mL of dry THF. PEG 200 (0.8 g, 4.0 mmol, 50 mol %) was added with stirring followed by addition of aminopropyl terminated polydimethylsiloxane (10 g, 4.0 mmol, 50 mol %). The resulting solution was warmed to 50° C. and dibutyltin bis(2-ethylhexanoate) (about 15 mg) was added. After an initial temperature rise to 83° C., the mixture was warmed and held at 70° C. for 12 hr, during which time the mixture had become very viscous. After cooling, the mixture was poured into 3 L of rapidly stirring DI water. The precipitated polymer was collected, washed with DI water (3×), torn into small pieces and dried at 50° C. until a constant weight was obtained.

A membrane was prepared as described above. An infrared spectrum of the product was obtained and is reproduced in FIG. **9**, exhibiting the expected absorbance bands (cm^{-1}).

Example 5

This example provides the formulations and properties of representative membranes.

Table 3 provides the five formulations for representative polymers which were then formed into membranes. The polymers were prepared by solution polymerization.

TABLE 3

Representative Polymer Formulations

Polymer	Diisocyanate	Poly (alkylene glycol)	Aliphatic diol	Siloxane
1	1,6-Hexamethylene	PEG 600 (20%)	DEG (60%)	Aminopropyl (20%)
2	Isophorone	PEG 600 (20%)	DEG (50%)	Aminopropyl (30%)
3	1,6-Hexamethylene	PEG 600 (50%)	None	Aminopropyl (50%)
4	1,6-Hexamethylene	PEG 400 (40%)	None	Aminopropyl (60%)
5	1,6-Hexamethylene	PEG 600 (60%)	None	Aminopropyl (40%)

Table 4 provides certain physical and chemical properties of the polymers provided above.

TABLE 4

Physical Properties of Representative Polymers

Polymer	Water Pickup (%)	D_{oxygen} ($\times 10^{-6}$ cm^2/sec)	$D_{glucose}$ ($\times 10^{-9}$ cm^2/sec)
1	28.5	1.21	18.5
2	31.3	0.57	55.7
3	44	1.50	105
4	57	1.22	13.5
5	71	1.45	155

Example 6

This example illustrates the evaluation of a membrane-coated biosensor constructed according to the present invention.

A membrane prepared from the polymer identified as 3 above was found to have excellent mechanical properties as well as appropriate oxygen and glucose diffusivities. The membrane was evaluated using a prototype glucose sensor illustrated in FIG. **10**A According to FIG. **10**A, a sensor **10** was constructed having a reference electrode **12**, a working electrode **14**, and a counter electrode **16** deposited on a polymeric sheet **19**. A series of bonding pads **18** complete the sensor **10**. As shown in FIG. **10**B, the working electrode **14** was covered with a layer **20** of the enzyme glucose oxidase and the entire electrode array was coated with a layer **22** of the polymer **3** by dip coating two times from a 5 wt % solution of the polymer in THF. The sensor was connected to a commercial potentiostat (BAS Instruments, not shown) and operated with a potential of +0.6 volts between the working electrode and the reference electrode.

Glucose response is shown in FIG. **11**. As seen in FIG. **11**, the response of the electrode system is linear over the physiological glucose range, suggesting relative independence of local O_2 concentration. All of the other polymers tested showed similar behavior to the polymer identified as 3 and are acceptable as membranes for biosensor applications.

The above description is illustrative and not restrictive. Many variations of the invention will become apparent to those of skill in the art upon review of this disclosure. Merely by way of example a variety of solvents, membrane formation methods, and other materials may be used without departing from the scope of the invention. The scope of the invention should, therefore, be determined not with refer-

ence to the above description, but instead should be determined with reference to the appended claims along with their full scope of equivalents.

All publications, patents and patent applications mentioned in this specification are herein incorporated by reference into the specification to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference.

What is claimed is:

1. A method for forming an implantable biosensor having a biocompatible coating, the method comprising coating the implantable biosensor with a hydrogel composition formed by admixing (a) a diisocyante, the diisocyanate comprising about 50 mol % of the reactants in the admixture; (b) a hydrophilic polymer selected from, the group consisting of a hydrophilic polymer diol, a hydrophilic polymer diamine and combinations thereof; and, optionally, (c) a chain extender, thereby forming the implantable biosensor having the hydrogel coating, wherein the hydrogel composition has a water pickup of at least about 120% by weight.

2. The method in accordance with claim **1**, wherein the coating is by spin coating, dipping or spraying.

3. The method in accordance with claim **1**, wherein the hydrogel composition has a water pickup of from about 120% to about 400% by weight.

4. The method in accordance with claim **1**, wherein the diisocyanate is a member selected from the group consisting of isophorone diisocyanate, 1,6-hexamethylene diisocyanate and 4,4'-methylenebis(cyclohexyl isocyanate).

5. The method in accordance with claim **1**, wherein the hydrophilic polymer diol is a member selected from the group consisting of a poly(alkylene)-glycol, a polyester-based diol and a polycarbonate polyol.

6. The method in accordance with claim **1**, wherein the hydrophilic polymer diamine is a diamino poly (oxyalkylene).

7. The method in accordance with claim **4**, wherein the diamino poly(oxyalkylene) is poly(propylene glycol) bis(2-aminopropyl ether).

8. The method in accordance with claim **4**, wherein the diamino poly(oxyalkylene) is a member selected from the group consisting of a polyoxypropylenediamine having an average molecular weight of about 230, a polyoxyethylene-diamine having an average molecular weight of about 600, a polyoxyethylenediamine having an average molecular weight of about 900 and a polyoxypropylenediamine having an average molecular weight of about 2000.

9. The method in accordance with claim **1**, wherein the chain extender is selected from the group consisting of an alkylene diol, an alkylene diamine, aminoalkanol and combinations thereof.

10. The method in accordance with claim **1**, wherein the chain extender is selected from the group consisting of butanediol, ethylenediamine; hexamethylenediamine, 1,2-diaminocyclohexane and isophoronediamine.

11. The method in accordance with claim **1**, wherein the diisocyanate is 1,6-hexamethylene diisocyanate and the hydrophilic polymer is selected from the group consisting of a polyoxyethylenediamine having an average molecular weight of about 600, a polyoxypropylenediamine having an average molecular weight of about 2000 and a poly(ethylene glycol) having in average molecular weight of about 2000 and is present in an amount of about 40 to about 50 mol %.

12. The method in accordance with claim **1**, wherein the diisocyanate is 1,6-hexamethylene diisocynate, the hydrophilic polymer is selected from the group consisting of a polyoxyethylenediamine having an average molecular weight of about 600, a polyoxypropylenediamine having an average molecular weight of about 2000 and a poly(ethylene glycol) having at avenge molecular weight of about 2000 and is present in amount of about 40 to about 50 mol %, and the chain extender is butanediol and is present in an amount of about 2.5 to about 10 mol %.

13. A method for forming an implantable biosensor having a biocompatible coating, the method comprising coating the implantable biosensor with biocompatible composition formed by admiring (a) a diisocyanate, the diisocyanate comprising about 50 mol % of the reactants in the mixture; (b) a hydrophilic polymer which is a member selected from the group consisting of a hydrophilic polymer diol, a hydrophilic polymer diamine and combinations thereof; (c) a siloxane polymer having a glucose groups at the chain termini, the biocompatible composition having a glucose diffusion coefficient of from about 1×10^{-9} cm^2/sec to about 200×10^{-9} cm^2/sec, a water pickup of at least 25% and a ratio of $D_{oxygen/glucose}$ of from about 5 to about 200.

14. The method in accordance with claim **13**, wherein the functional groups are members selected from the group consisting of amino, hydroxyl and carboxylic acid.

15. The method in accordance with claim **13**, wherein the hydrophilic polymer is a poly(ethylene)glycol selected from the group consisting of PEG 200, PEG 400 and PEG 600.

16. The method in accordance with claim **13**, wherein the diisocynate is a member selected horn the group consisting of isophorone diisocyanate, 1,6-hexamethylene diisocyanate and 4,4'-methylenebis(cyclohexyl isocyanate).

17. The method in accordance with claim **13**, wherein the reaction mixture further comprises (d) a chain extender.

18. The method in accordance with claim **17**, wherein the chain extender is selected from the group consisting of an alkylene diol, an alkylene diamine, an aminoalkanol and combinations thereof.

19. The method in accordance with claim **13**, wherein the diisocyanate is 1,6-hexamethylene diisocyanate, the hydrophilic polymer is selected from the group consisting of PEG 400 and PEG 600 and is present in an amount of about 17 to about 32 mol %, and the siloxane polymer is aminopropyl polysiloxane having a molecular weight of about 2000 to about 4000 and is present in an amount of about 17 to about 32 mol %.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,784,274 B2
DATED : August 31, 2004
INVENTOR(S) : William Peter Van Antwerp et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

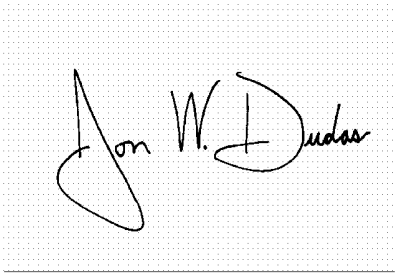
Title page,
Item [75], Inventors, "**Mastrototoro**" should read -- **Mastrototaro** --.

Column 19,
Line 16, after "from" delete the comma ",".

Column 20,
Line 7, "in" should read -- an --.
Line 10, "diisocynate" should read -- diisocyanate --.
Line 15, "at" should read -- an --, "avenge" should read -- average --.
Line 21, after "with" insert -- a --.
Line 22, "admiring" should read -- admixing --.
Line 27, "a glucose" should read -- functional --.
Line 39, "diisocynate" should read -- diisocyanate --.

Signed and Sealed this

First Day of February, 2005



JON W. DUDAS
Director of the United States Patent and Trademark Office

专利名称(译)	用于生物传感器的亲水性，可溶胀涂层		
公开(公告)号	US6784274	公开(公告)日	2004-08-31
申请号	US10/213265	申请日	2002-08-06
[标]申请(专利权)人(译)	VAN安特卫普威廉彼得 DECKER CHRISTIANÇ MASTROTOTARO JOHNĴ		
申请(专利权)人(译)	VAN安特卫普威廉彼得 DECKER CHRISTIAN C. MASTROTOTARO约翰J.		
当前申请(专利权)人(译)	MINIMED INC.		
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摘要(译)

提供了通过用均匀的水凝胶涂覆生物传感器的表面来降低可植入生物传感器的电极阻抗的方法，所述水凝胶允许在传感器周围无阻碍地移动水。表面涂层是生物相容的组合物，其吸水量至少为其重量的120％，更优选至少为其重量的200％。在吸收水时，本发明中使用的水凝胶也会膨胀并在水凝胶附着的电极周围提供一层水。水凝胶可由（a）二异氰酸酯，（b）亲水性聚合物（亲水性二醇，亲水性二胺或其组合）和任选的（c）增链剂制备。