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TITLE OF INVENTION

54 **ORGANIC ION-SELECTIVE MEMBRANES**

57 Abstract (not more than 150 words) and figure of the drawings to which the abstract refers, are attached.

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Abstract

The invention provides ion-selective membranes made without use of extraneous solvents, and methods of making such membranes. The membranes of the invention are less porous, more inert to water, have a longer shelf life, and are suitable for efficient manufacture in large quantities.

ORGANIC ION-SELECTIVE MEMBRANES

Background of the Invention

1. Field of the Invention

The present invention relates to ion-selective membranes and methods of their preparation. Specifically it relates to improved properties of polymer membranes that are formed without the use of solvents.

2. Discussion of the Background

Ion-selective membranes have uses in numerous applications, particularly in biosensors and analytical devices. Typically, such membranes are used to separate a test solution from a reference solution, allowing electrochemical measurements of the differences in ion concentration across the membrane. Recent theoretical advances have created prospects for a marked increase in the detection limits of such devices. Chemical and Engineering News, November 24, 1997, p. 13. However, presently available ion-selective membranes impose significant limitations on overall sensitivity. These membranes also have other characteristics that seriously limit their long-term stability in aqueous solutions.

A major use of ion-selective membranes is in the field of disposable biosensors. Optimally, membranes made for these devices would have a long shelf life, low detection limits, and could be manufactured rapidly and efficiently in large scale. However, because of the means by which conventional ion-selective membranes are made, such membranes have inherent limitations on shelf life, detection limits, and efficiency of high volume manufacture.

The stability problems of available ion-selective membranes are due in large measure to the tendency of such membranes to swell in the presence of water. Swelling not only distorts the structure of the membrane, but it also changes membrane permeability, and it can cause components of the membrane to leach out, further altering membrane permeability and distorting the concentrations of ions or other chemical entities in the area near the membrane. This type of distortion affects both the detection limits and the accuracy of electrochemical measurements.

Physically or chemically unstable membranes further limit the potential for development of implantable biosensors. The use of implantable devices to detect physiological states, such as, for example, blood glucose levels, is not feasible if the membranes in such devices are subject to significant distortion or degradation upon contact with aqueous solutions. Accordingly, there is a great need for ion-selective membranes that have long-term stability in contact with aqueous solutions.

Ion-selective membranes of the prior art are manufactured by dissolving the required components in a common solvent and then casting the mixture into a suitable tool to form it into the shape utilized in the electrode. There are many general problems associated with this technology.

For example, solvent evaporation must occur under very tightly controlled conditions. If the speed of evaporation is too fast, a crust builds up and bubbles form beneath the crust. The bubbles thus formed tend to isolate the surface area of the membrane from the potential that generates the transmitting function across the membrane.

This, of course, causes diminished sensitivity of the membrane and in extreme cases can result in membranes that are entirely useless for electrode devices.

Another disadvantage is that the casting process must be repeated many times to produce a reasonable thickness of membrane that can be handled with tools. This repeated process can increase the accumulation of bubbles with each repeated step, thus disabling an increasingly larger area of the membrane.

In many cases, finding a common solvent is difficult, if not impossible, because not all of the membrane components and additives that would be beneficial in combination are soluble in the same solvent. This is particularly relevant in automated production of precalibrated, disposable electrochemical sensors, where components and additives are required that are not typical for membranes used in laboratory ion-selective sensors.

Given that the various desirable components in an ion-selective membrane are not always soluble in a single solvent, occasionally solvent mixtures are used to create the solution from which the membranes are cast. However, mixed solvents create additional problems in the evaporating step, especially in the case of azeotropic mixtures having high boiling points. Further, since the evaporation of solvents requires energy, which is extracted from the vicinity of the membrane, it can cause cooling of the membrane. This temperature reduction can cause water vapor deposition, altering the membrane surface and inhibiting the adhesion of the next layer of membrane material to the previously deposited portion of the membrane. The traditional casting process also requires precise measurement of the volume of the solvent mixture and prevention of solvent evaporation from the casting solution prior to the deposition of each new layer, in order to avoid changes in viscosity and component concentrations in the mixture.

Some modifications to the process of membrane manufacture have been developed to address the problems associated with evaporation casting. In one such process, a glass ring is adhered to a glass or metal plate. A polymer/solvent solution is then poured into the ring. The ring is covered by a larger chamber and evaporation is allowed to proceed at a relatively slow rate. After the solvent evaporates, the membrane can be cut into discs with a punch.

Since, in this procedure, the evaporation process is slower and more controlled, it is not always necessary to form the membrane in several layers. However, the advantage of one-step casting of membranes in such a protocol is diminished by the disadvantage of slow solvent evaporation, cumbersome setup, and limits of the number of useful membrane disks that can be recovered from each round of the process.

The membranes utilized in the automated production of sensors must be very uniform within a production lot, must also have a long shelf life, and must perform in a predictable manner. Because of the numerous inherent problems in forming electrode membranes by a solvent evaporation process, manufacturers are forced to produce membranes in relatively small production lots. Making membranes in small lots reduces the waste associated with a failed process, but it also puts very restrictive limits on efficiency and production capacity in the membrane formation process.

Because of the great variability between different production lots in solvent-based membrane formulations, attempts at high volume production, such as in the manufacture of disposable sensors, can be very inefficient,

unproductive and costly. With all of the above disadvantages, the casting of solvent-based membranes for post-manufacture integration into disposable biosensors is not very feasible.

As an alternative, the membranes may be formed *in situ* from a dissolved membrane-forming mixture. However, this requires expensive equipment capable of repeatedly dispensing small amounts of high viscosity solutions, subsequent protection against water deposition, controlled evaporation of the solvent to minimize bubble formation, long parking time in the equipment, and excessive delay in the manufacturing process.

Indeed, the difficulties and inefficiencies of solvent-based ion-selective membranes place severe limits on the mass manufacture of disposable devices employing such membranes. This is the case because of the variability of the results, so that membranes formed for post-manufacture integration into biosensor devices must be produced in small lots to prevent waste of large lots of expensive materials. This is likewise true because membranes formed *in situ* on disposable medical devices create comparable inefficiencies in use of equipment.

A problem, perhaps even more severe, with solvent-based ion-selective membranes is the fact that such membranes are inherently unstable when in contact with aqueous solutions. This is because as the solvent leaves the membrane structure by evaporation, it leaves behind pores, channels, and other irregularities in the membrane structure. These artifacts of solvent evaporation are readily attacked by water, which causes rapid distortion of membrane structure and electrochemical function.

Therefore, it has been necessary to develop elaborate means of preventing water contact with prior art membranes in electrode devices until the very moment that such devices are to be used in a measurement. This is because typical prior art membranes absorb significant amounts of water within seconds of their first contact with an aqueous solution. The absorption of water changes membrane geometry, composition, and electrochemical function. For example, as water moves through an electrode membrane, the situs of the electrical potential is likewise in motion, and additives and ions previously immobilized within the membrane can begin to diffuse as well.

This phenomenon inhibits the formation of, and distorts the magnitude of, an electrical potential across the membrane, and can, within a short time, lead to a complete loss of potential as the aqueous solution moves through the membrane. This is because the infiltration of water into the membrane causes the movement and diffusion of previously immobilized active ingredients within the membrane, thus carrying with them the ions responsible for formation of the potential.

If these active ingredients move toward the outside of the membrane and release ions at the membrane surface, the released ions raise the local ion concentration outside the membrane and interfere with the detection limits of the electrode. If the active ingredients diffuse toward the reference solution side of the membrane, the ions previously immobilized within the membrane are depleted, and the ability to establish a potential across the membrane is reduced or destroyed. Because the electrochemical properties of the membrane can change over the course of a very few seconds, automated measurements of membrane potential must be done extremely rapidly, and inherently must involve approximations and extrapolations that are subject to significant error.

Accordingly, the disadvantages of solvent-based, ion-selective membranes include both inefficiencies of production and irregularities in performance. Such membranes have an inherently short shelf life, particularly in the presence of any form of humidity, and are thus not optimal for disposable biosensors that may require long shelf life. Of course such membranes are also wholly unsuitable for use in implantable biosensor devices, both because of their rapid physical and performance degradation in contact with aqueous solutions, and because of the biocompatibility problems commonly associated with implanted plastics. There is, therefore, a great need for new approaches to membrane manufacture and for improved ion-selective membranes having significantly reduced rates of water absorption, greatly simplified manufacturing requirements, and enhanced biocompatibility. Disclosed herein are membranes exhibiting highly desirable physical properties, as well as methods of their manufacture that represent a vast improvement over the methods disclosed in the prior art.

Summary of the Invention

The present invention provides a method of producing an ion-selective membrane. In the method, membrane components including a polymer and at least one additive are combined to form a mixture without addition of a solvent. A superior ion-selective membrane may be formed from this mixture. In this aspect of the invention, the polymer may be vinyl chloride. The polymer may also be polyvinyl chloride or it may be a copolymer of vinyl chloride, such as, for example vinyl acetate and/or vinyl alcohol. Also in this aspect of the invention, the additive may be a plasticizer. Suitable plasticizers may include, for example, one or more of the following: aromatic ethers, aliphatic-aromatic ethers, adipic acid esters, sebasic acid esters, phthalic acid esters, lauric acid esters, glutaric acid esters, and phosphoric acid esters. The additive may also be an ion-selective agent.

According to the method of the invention, the membrane components may include more than one additive. For example, the membrane components may include a plasticizer and an ion-selective agent. The membrane components further may include an additive such as, for example, plasticizer modifiers, active ingredients, ion mobility enhancers, heat stabilizers, light stabilizers, surface activity modifiers, lipophilizers, and intermediary immobilizers.

In the method of the invention the combining step may include mixing the components in a homogenizer. It may also include heating the components. The forming step of the method may include extruding the mixture onto a device adapted for use with the membrane. The membrane may also be formed by injection molding the mixture. In the practice of this method, the invention also contemplates reacting a biomolecule with the components of the membrane to form a bond between the biomolecule and the membrane at the membrane's surface. Biomolecules that may be thus reacted include, for example, enzymes, receptors, hormones, nucleic acids and antibodies.

The method of the invention may further include contacting a surface of the membrane with an aqueous material that may contain a biomolecule, to form a two-layer ion-selective membrane having a solvent-free layer and an aqueous layer. In this aspect of the invention, the biomolecules may be, for example, enzymes, receptors, hormones, nucleic acids and antibodies.

In another aspect of the invention is provided a solvent-free ion-selective membrane made of a polymer and at least one additive, wherein the membrane is formed without a solvent. The polymer of the membrane may be, for

example, vinyl chloride, polyvinyl chloride, or a copolymer of vinyl chloride, such as vinyl acetate and/or vinyl alcohol. The additive may be a plasticizer. Useful plasticizers include, for example, aromatic ethers, aliphatic-aromatic ethers, adipic acid esters, sebasic acid esters, phthalic acid esters, lauric acid esters, glutaric acid esters, and phosphoric acid esters. The additive also may be an ion-selective agent. Membrane components may also include more than one additive, such as a plasticizer and an ion-selective agent. The membrane components further may include one or more additives such as, for example, plasticizer modifiers, active ingredients, ion mobility enhancers, heat stabilizers, light stabilizers, surface activity modifiers, lipophilizers, and intermediary immobilizers.

The ion-selective membranes of this aspect of the invention may have a curing mass loss less than 10%, or in some embodiments less than 1%. The membranes may have a water absorption index less than 0.5%, or in some embodiments less than 0.1%. In some embodiments of the invention, a biomolecule may be bound to a surface of the membrane. Useful biomolecules may include, for example, enzymes, receptors, hormones, nucleic acids and antibodies. The membranes may also have a layer of aqueous material in contact with a surface of the membrane, and the aqueous material may contain a biomolecule.

In another aspect of the invention, there is provided an improved biosensor device including an electrode, wherein the electrode has an ion-selective membrane. In this aspect of the invention, the improvement is a solvent-free ion-selective membrane made up of a polymer and at least one additive, wherein the improved membrane is formed without a solvent.

Detailed Description of the Preferred Embodiment

The membranes of the present invention are formed without the use of extraneous solvents. An extraneous solvent is a solvent that is added to increase the solubility of the components, which solvent is later removed as part of the formation of the membrane. Because the membranes of the invention do not employ extraneous solvents, they do not require evaporation steps. And since these membranes are formed without evaporation of a solvent, they exhibit structural and physical properties that are distinct from prior art membranes. Among the most important of those properties is a greatly reduced degree and rate of water absorption. Because the membranes of the present invention do not readily absorb water, they are not subject to the rapid distortions in structure, composition, and electrochemical properties that have plagued membranes of the prior art. Rather, they are highly stable and exhibit much more reproducible electrochemical properties over a much longer period of time in contact with aqueous solutions. "Aqueous solutions" as discussed herein may include solutions with any appreciable water content, including, for example, beverages, pharmaceutical solutions or suspensions, culture or fermenter media, blood, plasma, urine, cerebrospinal fluid, mucous secretions, and the like.

In addition to their stability in contact with aqueous solutions, these membranes also have a very long shelf life, and devices employing these membranes do not require specialized packaging or other measures intended to protect the membranes from moisture or humidity. Further, membranes of the present invention are capable of lower limits of ion detection, more rapid response, more accurate and reproducible measurements over time, and greater mechanical strength, such as would be beneficial for use under positive pressure or vacuum. Additionally, because of

their greatly reduced water absorption, the membranes of the present invention are much more effective in immobilizing membrane components. That is, important additives in the membrane, as discussed below, do not tend to leach out of the membrane, as is commonly the case with membranes of the prior art.

The membranes of the present invention are made without solvents; they are instead made by combining a polymer, a plasticizer, and other optional ingredients, which are mixed mechanically to form a mixture for membrane formation. For example, mechanical mixing may employ a high speed blender or homogenizer, which is well known in the plastics industry. Components may be added individually or in groups into the base polymer. Such components can be plasticizers, heat stabilizers, UV absorbers, gamma ray stabilizers, anti static agents, ion-selective agents and other ligands, conductive agents, waxes, hydrophobic agents, flow reduction agents, and the like. Without the problems associated with solvent removal, membranes of the invention can be formed to virtually any desired thickness, eliminating the need to form membranes in a series of thin-layer depositions with controlled evaporation steps in between.

The solvent-free mixture of membrane components can be pre-granulated or it can be directly fed into an extruder or injection molding machine. With extrusion, a ribbon of nascent membrane material can be fed easily into high speed production equipment. In this manner, membranes with new characteristics or mixtures of characteristics may be produced, many of which mixtures were heretofore impossible with previously known ion-selective membrane technology. The thickness of the membrane may be controlled with tooling or extrusion dies. The membrane thus produced may be rolled in quantities for prolonged production shifts of days or weeks. Since there are no solvents involved, the membranes display many superior properties over prior art membranes discussed above. The per-unit production cost is very low, and high volume automated production is relatively simple.

It is noted that plasticizers are capable of solubilizing polymers in many cases. Accordingly, in some publications (e.g. Suzuki, et al., Anal. Chem. 1989, 61:382-384) plasticizers themselves have been misdesignated as solvents. More correctly, and for the purposes of this application, a distinction is drawn between a true solvent and a plasticizer in membrane production. A true (extraneous) solvent is used to dissolve the components of the membrane and is then, either in whole or in part, removed from the membrane as it cures. The removal of the solvent generally is accomplished by means of evaporation, the disadvantages of which have been discussed above. Examples of true solvents that commonly have been used in solvent-based polymeric membranes of the prior art include cyclohexanone, methylene chloride, propylene carbonate, tetrahydrofuran, toluene, methanol, and water. The membranes of the present invention are thus made without addition of these or any other extraneous solvents, and have the benefit of being highly resistant to degradation caused by contact with water or solutions containing water.

In contrast to true solvents, a plasticizer may also be used to dissolve membrane components, but it remains part of the membrane and does not require any evaporation steps, nor does it produce the structural artifacts of evaporation. To further draw this distinction, a solvent-based membrane solution that is cast to form a membrane will lose significant mass during the process of solvent evaporation. However, a solvent-free mixture used to form a membrane will not lose significant mass during the manufacturing process. Of course, much more important than

issues of loss of mass are the resultant disadvantages of solvent evaporation, such as structural distortions and membrane porosity, water deposition on the curing membrane, elaborate control of evaporation parameters and/or the long delays and equipment downtime associated with curing by means of evaporation.

Membranes of the invention, being solvent-free, permit greatly increased rates of production, require significantly less control over environmental variables such as humidity, avoid the expense and environmental regulation difficulties associated with using and removing large amounts of organic solvents, and ultimately produce membranes having a degree of water absorption that is a small fraction of the water absorption of membranes of the prior art. Water absorption by membranes of the present invention, compared with ion-selective membranes of the prior art, thus may be reduced by a factor of 5, 10, 100, or more.

The final density of a cured membrane provides a distinction between the solvent-based ion-selective membranes of the prior art and the solvent-free ion-selective membranes of the present invention. As discussed at length above, solvent evaporation creates artifacts in the membrane, causing undesirable irregularities of structure and membrane porosity. In contrast, solvent-free membranes do not display artifacts of evaporation, and are essentially non-porous.

In the filtration membrane art, bulk porosity is defined as the "dead space" within a membrane, and is calculated by weighing a membrane sample of known volume, and comparing its density with the overall density of the base polymer and any additives that may be present. For filtration membranes, high bulk porosity is a desirable condition. In contrast, ion-selective membranes would ideally approach zero bulk porosity--they are ideally non-porous. The difference between the ideal and the actual can be expressed as the Membrane Density Ratio (MDR). Solvent-based ion-selective membranes having bubbles or pores may have an MDR of 80% or less, while solvent-free membranes generally have an MDR greater than 85%, preferably greater than 95%, and most preferably greater than 99%.

The membranes of the present invention may contain a polymer, typically polyvinylchloride (PVC) or a PVC copolymer; plasticizers; plasticizer modifiers; active ingredients; ion mobility enhancers; heat stabilizers; light stabilizers; surface activity modifiers; lipophilizers; intermediary immobilizers; and/or other components. PVC polymers or copolymers include low, medium, high and ultra-high molecular weight PVC. Also useful are copolymers of vinyl chloride such as vinyl chloride/vinyl acetate, vinyl chloride/vinyl alcohol, and vinyl chloride/vinyl acetate/vinyl alcohol. Another suitable polymer is carboxylated polyvinyl chloride.

Depending on the formulation, any of several known plasticizers may be used. Examples of appropriate plasticizers are aromatic ethers; aliphatic-aromatic ethers; esters of adipic acid, sebasic acid, phosphoric acid, glutaric acid, phthalic acid, and/or lauric acid; and long chain aliphatic alcohols. Non-limiting examples of plasticizer modifiers are halogenated paraffins such as chloroparaffins, long chain aliphatic alcohols, substituted nitrobenzenes, and acetophenones. Active ingredients that may be used in membranes of the present invention include, among others, antibiotics, liquid ion exchangers, neutral carriers, substituted amines, organo-ammonium salts, crown ethers, hormones, enzymes, antigens, antibodies, DNA binding factors, nucleic acids, and the like. Ion mobility enhancers

include, for example, salts of stearic acid, long chain alcohols, and waxes including but not limited to paraffin waxes. Examples of useful heat stabilizers are salts of stearic acid, organo-metallic compounds, and chlorine receptors. Light stabilizers can include both UV absorbers and light-to-heat converters. Examples of surface activity modifiers are cellulose triacetate, polyacrylamide, organo-ammonium salts, and the like.

While the possible active ingredients are far too many to list exhaustively, several particularly useful ion-selective agents are listed in Table 1. It will be recognized by those of skill in the art that other ion-selective agents, as well as other types of active ingredients, can be selected and employed in the membranes of the invention, to optimize membrane function based on the particular desired use and properties of the membrane.

Table 1. Examples of ion-selective agents

Ion or other Analyte	Selective Agent(s)
ammonium	nonactin
calcium	calcimycin (N,N,N',N'-tetracyclohexyl-3-oxapentanediamide) (-)-(R,R)-N,N'-[bis(11-(ethoxycarbonyl)undecyl)]-N,N'-4,5-tetramethyl-3,6-dioxaoctane-diamide N,N-dicyclohexyl-N',N'-dioctadecyl-3-oxapentanediamide bis[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphate calcium salt
carbonate	heptyl 4-trifluoroacetylbenzoate 1-(dodecylsulfonyl)-4-trifluoroacetylbenzene N-dodecyl-4-trifluoroacetylacetanilide 4-butyl-α,α,α-trifluoroacetophenone
chloride	tridodecylmethylammonium chloride 5-10-15-20-tetraphenyl-21H,23H-porphyrin manganese(III) chloride 4,5-dimethyl-3,6-dioctyloxy-1,2-phenylene-bis (mercury-trifluoroacetate)
hydrogen	tridodecylamine 4-nonadecylpyridine octadecyl isonicotinate
lithium	N,N,N',N'-tetraisobutyl-cis-cyclohexane-1,2-dicarboxamide N,N-dicyclohexyl-N',N'-disisobutyl-cis-cyclohaxane-1,2-dicarboxamide 5-butyl-5-ethyl-N,N,N',N'-tetracyclohexyl-3,7-dioxaazelaic diamide 12-crown-4; 1,4,7,10-tetraoxacyclododecane 6,6-dibenzyl-14-crown-4 6-[2-(diethylphosphonooxy)-ethyl]-6-dodecyl-14-crown-4 N,N,N',N',N'',N''-hexacyclohexyl-4,4',4''-propylidyne-tris-(3-oxabutyramide)
magnesium	N,N'-diheptyl-N,N'-dimethyl-1,4-butanediamide N,N''-octamethylen-bis(N'-heptyl-N'-methyl-methylmalonamide N,N''-octamethylene-bis(N'-heptyl-N'-methylmalonamide) N,N'N''-tris[3-(heptylmethylamino)-3-oxopropionyl]-8,8'-iminodioctylamine
potassium	valinomycin bis[(benzo-15-crown-5)-4'-ylmethyl]pimelate 2-dodecyl-2-methyl-1,3-propanediyl-bis [N-(5'-nitro(benzo-15-crown-5)-4'-yl)carbamate]
sodium	(N,N',N''-triheptyl-N,N',N''-trimethyl-4,4',4''-propylidyne-tris(3-oxabutyramide) N,N'-dibenzyl-N,N'-diphenyl-1,2-phenylendioxydiacetamide N,N,N',N'-tetracyclohexyl-1,2-phenylendioxydiacetamide 4-octadecanoyloxymethyl-N,N,N',N'-tetracyclohexyl-1,2-phenylendioxydiacetamide

Ion or other Analyte	Selective Agent(s)
sodium (cont.)	bis[(12-crown-4)methyl] dodecylmethylmalonate 4-tert-butylcalix[4]arene-tetraacetic acid tetraethylester decyl monensin
urea	urease enzyme/ammonium ion
glucose	glucose oxidase/oxygen

The membrane components are mixed by combining the polymer, the plasticizer, and any other optional additives. In some formulations, a plasticizer may also function as an active ingredient, or an active ingredient may function as a plasticizer. Thus, the invention contemplates solvent-free membranes made from as few as two components, as well as membranes made from a combination of numerous components.

Mixing may be facilitated with a high speed blender or by heat gelation and diffusion. Mixing may occur at ambient temperature, or an alternative temperature may be selected based on the particular combination and properties of the components. For example, where antibodies or other proteins are among the additives to the membrane, elevated temperatures may cause problems of protein denaturation, and would therefore dictate limits on the temperature that could be used in forming the membrane. Other considerations in selecting membrane formation temperature include the desired working viscosity of the membrane mixture, temperature tolerances of other components of disposable devices onto which the membranes may be formed, and the like.

The membranes may be formed by extrusion and stamping, including *in situ* extrusion directly onto an electrode device. The membranes may also be formed by injection molding or capillary extrusion, either *in situ* onto a device, or in a separate fabrication step. Additional means of membrane formation include, for example, vacuum forming, vacuum molding, compression molding, blow molding, and calendaring. Membranes may be formed to virtually any useful thickness.

The invention also contemplates solvent-free membranes as described herein with aqueous-active molecules adhered or bound to their surface. For example, catalysts, including enzymes, may function at the surface of the membrane to modify a chemical species which is not directly detectable into a product which can be detected with the electrode of the device. In other embodiments of the invention, other molecules, structures, or complexes, may function at the membrane surface to enhance or modify the function of the membrane or the electrode device as a whole. Advantageous molecules, structures, or complexes include, for example, hormones, antibodies, antigens, nucleic acids, receptors, binding proteins, pharmaceutical preparations, crystalline substances, and the like.

For example, an enzyme, such as urease, may be bound or attached to the surface of a solvent-free membrane. This modification allows a membrane electrode that is capable of detecting an ionic species such as ammonium to also indirectly detect a non-ionic species such as urea. That is, as urea molecules in a test solution come into contact with the urease enzyme immobilized at the surface of the solvent-free membrane, the enzyme catalyzes the breakdown of urea to produce ammonium ions, which are detected by the electrode.

Likewise, a receptor complex with ATPase activity may be immobilized at the surface of a solvent-free membrane sensitive to phosphate ions, to indirectly measure presence of the receptor's ligand. As the ligand binds the receptor, phosphate ions are released from ATP and these ions are detected by the electrode. This type of indirect detection of analytes assisted by soluble biomolecules held at or near the surface of a solvent-free membrane has broad applicability that will be appreciated by those of skill in the art.

There are numerous ways to form membranes having biomolecules or other desirable molecules or structures at their surface. In one embodiment of the invention, a solvent-free membrane is formed as disclosed herein. Subsequent to membrane formation, a selected molecule or structure is chemically linked to the membrane via active moieties within the polymer or co-polymer of the membrane. For example, an amine group may be reacted to a chlorine group on a polyvinyl chloride membrane in a coupling reaction driven by silver ions as a catalyst.

In this embodiment, covalent bonds are formed between the biomolecule and the polymer of which the membrane is formed. In another embodiment, one or more selected biomolecules are solubilized in water, and then a water soluble polymerizing agent is added to form a solution. The solution is contacted in a thin layer with a pre-formed solvent-free membrane according to the invention. Upon or after contact between the aqueous solution and the solvent-free membrane, the aqueous layer polymerizes and forms a thin layer bound to the solvent-free membrane, which thin layer immobilizes the biomolecules or other structures it contains.

In another embodiment of the invention, two separate membranes are formed. The first is a solvent-free membrane as described extensively herein. The second is a water-based membrane containing one or more water soluble active ingredients, such as biomolecules or other complexes or structures. The thickness of the water-based membrane would typically be 1 to 100 microns, although a membrane layer of any useful thickness may be applied to the solvent-free membrane. When the water-based membrane comes into contact with the solvent-free membrane it approximates and immobilizes the biomolecules or other complexes or structures at or near the surface of the solvent-free membrane.

In many of these embodiments, the end result is a membrane having the desirable water-excluding properties of the solvent-free membranes of the invention, while also having immobilized thereon molecules that may require water to perform their desired function. Accordingly, the invention is not limited merely to solvent-free membranes that may contain one or more active ingredients, but also encompasses solvent-free membranes that may bear one or more desirable water soluble molecules on their surface. The water soluble molecules thus immobilized may function as enzymatic catalysts to convert a non-measurable species into a measurable species. They may also perform other useful functions, such as concentration of desirable particles, exclusion of undesirable particles, initiation of biochemical pathways leading to a desirable and/or measurable product, and the like.

Examples

Representative solvent-free membranes were prepared for comparison with prior art solvent-based membranes. Samples of membranes selective for calcium, potassium, and ammonium were tested.

Example 1. Calcium-selective solvent-free membrane

A solvent-free membrane, selective for calcium ions, was prepared according to the following formulation: PVC, ultra high molecular weight, 100.00 parts; 2-nitrophenyl octyl ether, 250.00 parts; (-)-(R,R)-N,N'-(bis(11-ethoxycarbonyl)undecyl)-N,N'-4,5-tetramethyl-3,6-dioxaoctanediamide (calcium ion selective ligand), 7.50 parts; sodium tetraphenylborate, 1.50 parts; calcium stearate, 1.00 parts. All components were mixed to homogeneity and the membrane was formed to a thickness of about 1-3 mils (25-75 microns). After 3 h at 75-105°C, the membrane was fully solidified and functionally selective for calcium ions. Membranes of this formulation were then compared with conventional calcium-selective membranes. The results of such comparisons are provided and discussed below.

Comparative Example 1. Conventional calcium-selective membrane

A conventional formulation was used to prepare a solvent-based calcium-selective membrane for comparison to the calcium-selective membrane of the invention. The membrane was made using: N,N,N',N'-tetracyclo-3-oxapentanediamide, 10 mg; 2-nitrophenyl-octyl ether, 655 mg; potassium tetrakis(4-chlorophenyl)borate, 6 mg; PVC, high molecular weight, 328 mg. All components were dissolved in 8.0 ml (11,256 mg) tetrahydrofuran. Thus, the total mass of the formulation, prior to evaporation of the solvent, was 12,255 mg--about 8% solids and about 92% solvent. The membranes were cast and cured using conventional techniques, and the resulting membranes were functionally selective for calcium ions.

It is possible to prepare this type of conventional membrane using more or less solvent than the amount used in this comparative example, within a limited range. The optimal amount of solvent to be used is based on the desired viscosity of the solution, which is governed by the membrane manufacturing parameters. Solutions prepared with too little solvent are typically too viscous to dispense and do not properly settle into uniform, flat layers. Solutions containing excessive amounts of solvent are much less viscous, but require longer evaporation times and generally also require more dispensing and curing steps to achieve a specified membrane thickness.

The viscosity of the solution used in this comparative example, and the other comparative examples herein, was about equal to the viscosity of solutions that would be used in typical manufacturing processes of these conventional membranes. Thus, measures of mass or volume lost during curing of the membranes of the comparative examples are representative of the mass or volume loss in conventional, solvent-based, ion-selective membrane manufacture.

Example 2. Potassium-selective solvent-free membrane

A solvent-free membrane, selective for potassium ions, was prepared according to the following formulation: PVC, ultra high molecular weight, 100.00 parts; dioctyl adipate, 150.00 parts; valinomycin (potassium ion selective ligand), 2.85 parts; sodium tetraphenylborate, 2.00 parts; stearic acid, 1.00 parts. All components were mixed to homogeneity and the membrane was formed to a thickness of about 1-3 mils (25-75 microns). After 6 h at 85-115°C, the membrane was fully solidified and functionally selective for potassium ions. Membranes of this formulation were then compared with conventional potassium-selective membranes. The results of such comparisons are provided and discussed below.

Comparative Example 2. Conventional potassium-selective membrane

A conventional formulation was used to prepare a solvent-based potassium-selective membrane for comparison to the potassium-selective membrane of the invention. The membrane was made using: valinomycin, 10 mg; bis(1-butylpentyl) decane-1,10-diyl diglutarate, 650 mg; potassium tetrakis(4-chlorophenyl)borate; 5 mg; PVC, high molecular weight, 330 mg. All components were dissolved in 8.0 ml (11,256 mg) tetrahydrofuran. Thus, the total mass of the formulation, prior to evaporation of the solvent, was 12,251 mg--about 8% solids and about 92% solvent. The membranes were cast and cured using conventional techniques, and the resulting membranes were functionally selective for potassium ions.

Example 3. Ammonium-selective solvent-free membrane

A solvent-free membrane, selective for ammonium ions, was prepared according to the following formulation: PVC, ultra high molecular weight, 100.00 parts; bis(ethylhexyl) adipate, 250.00 parts; nonactin (ammonium ion selective ligand), 1.50 parts; ammonium tetraphenyl borate, 0.36 parts; stearic acid, 1.00 parts. All components were mixed to homogeneity and the membrane was formed to a thickness of about 1-3 mils (25-75 microns). After 6 h at 85-105°C, the membrane was fully solidified and functionally selective for Ammonium ions. Membranes of this formulation were then compared with conventional ammonium-selective membranes. The results of such comparisons are provided and discussed below.

Comparative Example 3. Conventional ammonium-selective membrane

A conventional formulation was used to prepare a solvent-based ammonium-selective membrane for comparison to the ammonium-selective membrane of the invention. The membrane was made using: nonactin, 10 mg; bis(butylpentyl)adipate, 668 mg; PVC, high molecular weight, 322 mg. All components were dissolved in 8.0 ml (11,256 mg) tetrahydrofuran. Thus, the total mass of the formulation, prior to evaporation of the solvent, was 12,256 mg--about 8% solids and about 92% solvent. The membranes were cast and cured using conventional techniques, and the resulting membranes were functionally selective for ammonium ions.

Example 4. Curing Mass Loss

The membranes of the invention do not lose significant mass during curing (or solidification), because they do not contain a solvent. In contrast, prior art ion-selective membranes, since they are formed using solvents, lose substantial mass as the solvent evaporates. Curing Mass Loss (CML) is a numerical expression of the percent loss of mass of the membrane solution during curing, and thus provides a meaningful and measurable distinction between the membranes of the invention and those of the prior art.

CML can be calculated based on the relative concentrations of the components in the solvent-based mixture by subtracting the percentage concentration of the solvent from 100. This of course assumes complete solvent removal. CML also may be determined empirically by weighing a given quantity of solvent-based membrane solution before casting, and later weighing the fully cured membrane produced therefrom. Table 2 provides calculated CML for prior art membranes and for solvent-free membranes of the invention.

Theoretical CML of the membranes of the present invention is 0%, and measured CML is generally less than 10%, preferably less than 1%, and most preferably less than 0.1%.

Table 2. Curing Mass Loss (CML)

Membrane	CML (%)
Solvent-free calcium-selective membrane	< 0.1
Conventional calcium-selective membrane	~ 92
Solvent-free potassium selective membrane	< 0.1
Conventional potassium-selective membrane	~ 92
Solvent-free ammonium selective membrane	< 0.1
Conventional ammonium-selective membrane	~ 92

Similar to mass loss is volume loss of a membrane as it solidifies. Membranes of the present invention exhibit little, if any, change in volume after solidification. In contrast, the membranes of the comparative examples exhibited volume loss of 91.6% (potassium-sensitive membrane) and 89.3% (ammonium-sensitive membrane). Volume loss was not measured for the conventional calcium-sensitive membrane.

Example 5. Water Absorption Index

Membranes of the present invention differ significantly from prior art organic ion-selective membranes because of their greatly reduced tendency to absorb water. The functional importance of this difference has been discussed above. A useful measure of this difference is the water absorption index (WAI). WAI is determined by placing a sample of membrane having a known mass into distilled water for 24h at room temperature. After 24h, the sample is re-weighed and the mass gain, if any, is expressed as a percentage of the original mass of the membrane. Different membranes then may be compared based on the WAI of each.

While WAI is an arbitrarily selected measure of water absorption, it is a simple, universal test that is clearly proportional to and indicative of differences in water affinities of different membranes. Depending on the intended uses of particular organic ion-selective membranes, other measures similar to WAI, as defined above, may also be useful.

For example, an absorption index using blood plasma as the aqueous solution, at body temperature, for a one week duration, would fairly indicate the degree to which membranes of distinct formulations would distort during one week in an implanted biosensor electrode. Tests of membranes intended to be selective for a particular ion also may be conducted using a solution containing the ion and, in many cases, other additives such as chelators of undesirable ions, pH buffers, and/or solution preservatives. While WAI parameters provide a simple, universal basis for comparison, other solutions more specific for the membranes in question may be used in comparison tests to accelerate water absorption, allowing more rapid detection of differences between membranes. See Example 7.

Membranes of the present invention absorb water much less than organic ion-selective membranes of the prior art, and are therefore much more stable in contact with aqueous solutions, as shown below in Table 3. Typically,

membranes of the invention have a WAI of less than 0.5%, preferably less than 0.1%, and most preferably less than 0.05%.

Table 3. Water Absorption Index (WAI)

Membrane	WAI (%)
Solvent-free calcium-selective membrane	< 0.1
Conventional calcium-selective membrane	0.69
Solvent-free potassium selective membrane	< 0.2
Conventional potassium-selective membrane	0.95
Solvent-free ammonium selective membrane	< 0.1
Conventional ammonium-selective membrane	0.72

To clarify the interpretation of the results presented in Table 3, if 1000 mg of dry membrane were used as starting material, the membranes of the invention would weigh between 1000 and 1002 mg after 24h in water, while a conventional potassium-selective membrane would weigh 1009.5 mg after 24h in water.

Example 6. Water Mass Loss

Another benefit of the membranes of the invention is that, since they do not absorb water to an appreciable degree, they likewise are not subject to loss of membrane components due to the leaching effects caused by water absorption and/or permeability. In contrast, conventional solvent-based ion-selective membranes actually lose mass, presumably due to the leaching out of internal membrane components during 24h contact with water.

As a subsequent procedure to the WAI test described in Example 5, membranes that had been soaked in water for 24h and surface dried where then air dried for 24h to remove residual water. The dried membranes were then re-weighed, and the mass of each soaked and dried membrane was compared with its original mass before exposure to water. As shown in Table 4, conventional membranes were shown to have lost mass, while the membranes of the invention exhibited no significant change in mass.

Table 4. Water Mass Loss (WML)

Membrane	WML (%)
Solvent-free calcium-selective membrane	< 0.1
Conventional calcium-selective membrane	0.45
Solvent-free potassium selective membrane	< 0.2
Conventional potassium-selective membrane	0.61
Solvent-free ammonium selective membrane	< 0.1
Conventional ammonium-selective membrane	0.54

To clarify the interpretation of the results presented in Table 4, if 1000 mg of dry membrane were used as starting material, the membranes of the invention would weigh between 1000 and 998 mg after 24h in water followed

by 24h of air drying, while a conventional potassium-selective membrane would weigh 993.9 mg after 24h in water followed by 24h of air drying.

Example 7. Ion Solution Absorption

Perhaps even more meaningful than the WAI, as a measure of the distortion of prior art membranes by the solutions they measure, is the membranes' absorption of solutions containing the target ions of those membranes. That is, a conventional calcium-selective membrane exhibits a greater mass gain when in contact with a calcium solution than when in contact with deionized water.

This is presumed to be due to the affinity of the ions in solution with the ion-selective agents in the membrane. As a membrane absorbs water, it is believed that ions also enter the membrane and interact with the active ingredients of the membrane. Internal ion accumulation can then lead to greater water accumulation as well. The result is that the membranes absorb the solution and become structurally distorted and functionally impaired. In contrast, the membranes of the examples do not exhibit significant ion solution absorption. In fact, the solvent-free membranes of the invention show no measurable difference in fluid absorption whether in contact with ion solutions or deionized water.

Ion solution absorption was determined by placing membrane samples in solutions containing 4 m eq of the ion for which the membrane is selective. Membranes were left in the ion solution for 24h at room temperature, then were blotted dry and weighed. Prior art membranes exhibited significantly increased mass, while mass increases in the membranes of the invention were at or near detection limits.

Table 5. Ion Solution Absorption (ISA)

Membrane	ISA (%)
Solvent-free calcium-selective membrane	< 0.1
Conventional calcium-selective membrane	2.37
Solvent-free potassium selective membrane	< 0.2
Conventional potassium-selective membrane	1.83
Solvent-free ammonium selective membrane	< 0.1
Conventional ammonium-selective membrane	1.16

Example 8. Ion Solution Retention

After the ion solution absorption experiment of Example 7, the membranes were air dried for 24h and re-weighed. Conventional membranes retained mass from the ion solution even after 24 of drying, while membranes of the invention showed no significant change in mass.

Table 6. Ion Solution Retention (ISR)

Membrane	ISR (%)
Solvent-free calcium-selective membrane	< 0.1

Membrane	ISR (%)
Conventional calcium-selective membrane	0.39
Solvent-free potassium selective membrane	< 0.2
Conventional potassium-selective membrane	0.47
Solvent-free ammonium selective membrane	< 0.1
Conventional ammonium-selective membrane	0.47

The foregoing examples are provided to demonstrate the significant advances in the ion-selective membrane art achieved by the present invention, and are merely representative of some embodiments of the invention. The true scope of the present invention is to be measured only by the following claims.

"Comprises/comprising" when used in this specification is taken to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps or components or groups thereof.

WHAT IS CLAIMED IS:

1. A method of producing an ion-selective membrane, comprising the steps of:

providing membrane components comprising a polymer and at least one additive that provides ion-selective properties;

combining said components to form a mixture without addition of a solvent; and

forming an ion-selective membrane from said mixture.

2. The method of Claim 1, wherein said polymer comprises vinyl chloride.

3. The method of Claim 2, wherein said polymer is polyvinyl chloride.

4. The method of Claim 2, wherein said polymer is a copolymer of vinyl chloride.

5. The method of Claim 4, wherein said copolymer comprises vinyl acetate.

6. The method of Claim 4, wherein said copolymer comprises vinyl alcohol.

7. The method of Claim 1, wherein said additive is a plasticizer.

8. The method of Claim 7, wherein said plasticizer is selected from the group consisting of aromatic ethers, aliphatic-aromatic ethers, adipic acid esters, sebasic acid esters, phthalic acid esters, lauric acid esters, glutaric acid esters, and phosphoric acid esters.

9. The method of Claim 1, wherein said additive is an ion-selective agent.

10. The method of Claim 1, wherein said membrane components comprise more than one additive.

11. The method of Claim 10, wherein said membrane components comprise a plasticizer and an ion-selective agent.

12. The method of Claim 11, wherein said membrane components further comprise an additive selected from the group consisting of plasticizer modifiers, active ingredients, ion mobility enhancers, heat stabilizers, light stabilizers, surface activity modifiers, lipophilizers, and intermediary immobilizers.

13. The method of Claim 1, wherein said combining step comprises mixing said components in a homogenizer.

14. The method of Claim 1, wherein said combining step comprises heating said components.

15. The method of Claim 1, wherein said forming step comprises extruding said mixture onto a device adapted for use with said membrane.

16. The method of Claim 1, wherein said forming step comprises injection molding said mixture to form said membrane.

17. The method of Claim 1, further comprising:

reacting a biomolecule with said components to form a bond between said biomolecule and said membrane at a surface of said membrane.

18. The method of Claim 17, wherein said biomolecule is selected from the group consisting of enzymes, receptors, hormones, nucleic acids and antibodies.

19. The method of Claim 1, further comprising:

contacting a surface of said membrane with an aqueous material, said aqueous material comprising a biomolecule, to form a two-layer ion-selective membrane comprising a solvent-free layer and an aqueous layer.

20. The method of Claim 19, wherein said biomolecule is selected from the group consisting of enzymes, receptors, hormones, nucleic acids and antibodies.

21. A solvent-free ion-selective membrane, said membrane comprising membrane components, said components comprising a polymer and at least one additive that provides ion-selective properties, said membrane being formed without a solvent.

22. The membrane of Claim 21, wherein said polymer comprises vinyl chloride.

23. The membrane of Claim 22, wherein said polymer is polyvinyl chloride.

24. The membrane of Claim 22, wherein said polymer is a copolymer of vinyl chloride.

25. The membrane of Claim 24, wherein said copolymer comprises vinyl acetate.

26. The membrane of Claim 24, wherein said copolymer comprises vinyl alcohol.

27. The membrane of Claim 25, wherein said additive is a plasticizer.

28. The membrane of Claim 27, wherein said plasticizer is selected from the group consisting of aromatic ethers, aliphatic-aromatic ethers, adipic acid esters, sebasic acid esters, phthalic acid esters, lauric acid esters, glutaric acid esters, and phosphoric acid esters.

29. The membrane of Claim 21, wherein said additive is an ion-selective agent.

30. The membrane of Claim 21, wherein said membrane components comprise more than one additive.

31. The membrane of Claim 30, wherein said membrane components comprise a plasticizer and an ion-selective agent.

32. The membrane of Claim 31, wherein said membrane components further comprise an additive selected from the group consisting of plasticizer modifiers, active ingredients, ion mobility enhancers, heat stabilizers, light stabilizers, surface activity modifiers, lipophilizers, and intermediary immobilizers.

33. The membrane of Claim 21, having a curing mass loss less than 10%.

34. The membrane of Claim 33, having a curing mass loss less than 1%.

35. The membrane of Claim 21, having a water absorption index less than 0.5%.

36. The membrane of Claim 35, having a water absorption index less than 0.1%.

37. The membrane of Claim 21, further comprising a biomolecule bound to a surface of said membrane.

38. The membrane of Claim 37, wherein said biomolecule is selected from the group consisting of enzymes, receptors, hormones, nucleic acids and antibodies.

39. The membrane of Claim 21, further comprising a layer of aqueous material in contact with a surface of said membrane, said aqueous material comprising a biomolecule.

40. The membrane of Claim 39, wherein said biomolecule is selected from the group consisting of enzymes, receptors, hormones, nucleic acids and antibodies.

41. An improved biosensor device comprising an electrode, said electrode comprising an ion-selective membrane, the improvement comprising:

a solvent-free ion-selective membrane, said membrane comprising membrane components, said components comprising a polymer and at least one additive that provides ion-selective properties, said membrane being formed without a solvent.

42. A method according to the invention for producing an ion-selective membrane, substantially as hereinbefore described and exemplified.

43. A method for producing an ion selective membrane including any new inventive integer or combination of integers, substantially as herein described.

44. A solvent-free ion-selective membrane as claimed in any of claims 21 to 40, substantially as hereinbefore described and exemplified.

45. A solvent-free ion-selective membrane including any new inventive integer or combination of integers, substantially as herein described.

46. An improved biosensor device as claimed in claim 41, substantially as hereinbefore described and exemplified.

47. An improved biosensor device including any new inventive integer or combination of integers, substantially as herein described.