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(54) **Title:** REFERENCE ELECTRODES INCLUDING SILICONE-CONTAINING POLYMER AND IONIC LIQUID

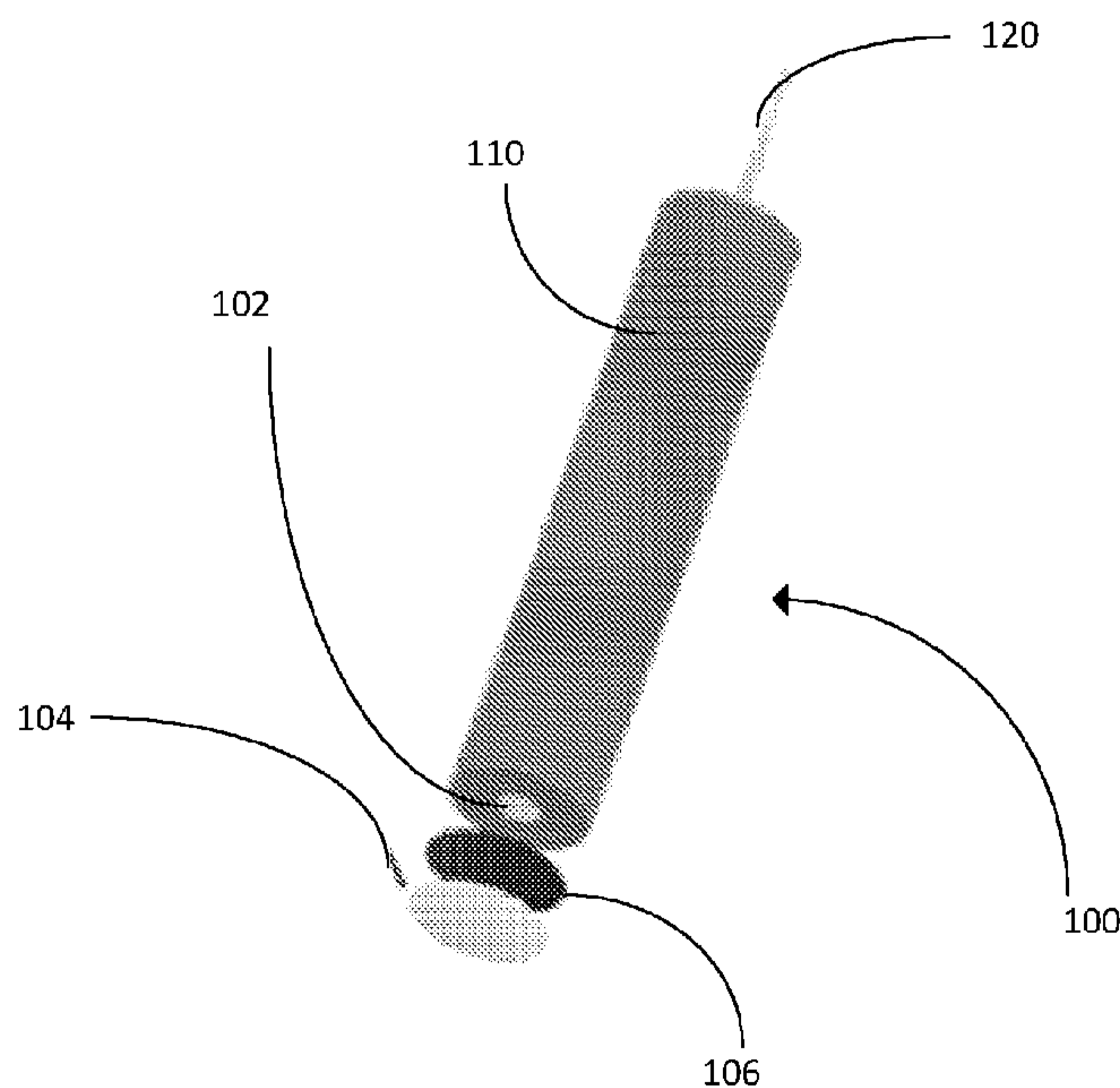


Figure 1

(57) **Abstract:** Reference electrodes that include a solid electron conductor; an interlayer; and a polymeric membrane layer comprising a polymer composition, the polymer composition comprising at least one silicone-containing polymer and at least one ionic liquid, wherein the polymeric membrane layer and the polymer composition are essentially plasticizer free and the silicone-containing polymer and the ionic liquid are miscible as determined using a thermodynamic method that shows a single glass transition temperature (T_g) of a composition including the at least one silicone-containing polymer and the at least one ionic liquid, wherein the interlayer is disposed between the solid electron conductor and the polymeric membrane layer. Also disclosed are electrochemical measurement systems including such reference electrodes.



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REFERENCE ELECTRODES INCLUDING SILICONE-CONTAINING POLYMER AND
IONIC LIQUID

RELATED APPLICATIONS

- [01] The present application claims the benefit of U.S. Provisional Application No. 62/933,608, filed November 11, 2019, which is incorporated herein by reference in its entirety.

SUMMARY

- [02] Disclosed herein are reference electrodes that include a solid electron conductor, an interlayer, and a polymeric membrane layer comprising a polymer composition, the polymer composition comprising at least one silicone-containing polymer and at least one ionic liquid, wherein the polymeric membrane layer and the polymer composition are essentially plasticizer free and the silicone-containing polymer and the ionic liquid are miscible as determined using a thermodynamic method that shows a single glass transition temperature (T_g) of a composition including the at least one silicone-containing polymer and the at least one ionic liquid, wherein the interlayer is disposed between the solid electron conductor and the polymeric membrane layer.
- [03] Also disclosed are electrochemical measurement systems including such reference electrodes.
- [04] The above summary of the present disclosure is not intended to describe each disclosed embodiment or every implementation of the present disclosure. The description that follows more particularly exemplifies illustrative embodiments. In several places throughout the application, guidance is provided through lists of examples, which examples can be used in

various combinations. In each instance, the recited list serves only as a representative group and should not be interpreted as an exclusive list.

BRIEF DESCRIPTION OF THE FIGURES

- [05] Figure 1 is a schematic depiction of an illustrative reference electrode.
- [06] Figure 2 shows the effect of KCl (1.0 to 16 mM) on the potential of reference electrodes with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -doped silicone membranes (conventional electrode setup with a 1.0 mM KCl inner filling solution), as measured against a free-flow double-junction reference electrode.
- [07] Figure 3 shows the potential responses of $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -doped Fluorosilicone 1 reference electrodes to artificial blood electrolyte solutions of high and low concentrations (see Table 3 for the composition of test solutions). Solid contact electrodes were prepared with CIM carbon as transducer layer, and conventional electrode bodies contained 1.0 mM KCl inner filling solution. Potentials were measured against a free-flow double-junction reference electrode. The average slopes of conventional electrodes and solid-contact electrodes are -0.01 ± 0.002 mV/decade ($n=3$) and -0.1 ± 0.2 mV/decade ($n=3$), respectively.
- [08] Figure 4 shows the long term emf as observed with a $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -doped Fluorosilicone 1 solid-contact reference electrode immersed into artificial blood electrolyte solutions (a 1:1 mixture of the HIGH and LOW solutions described by Table 3) against a free-flow double-junction reference electrode at 37 °C. (The data highlighted in red was used for the linear fit shown in black.).
- [09] Figure 5 shows the potential responses of reference electrodes with Fluorosilicone 1 membranes doped with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$, $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$, $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$, or $[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$ in the

1.0 to 16 mM KCl range. Average slopes and standard deviations of linear regressions are shown in Table 4.

[010] The figures are not necessarily to scale. Like numbers used in the figures refer to like components. However, it will be understood that the use of a number to refer to a component in a given figure is not intended to limit the component in another figure labeled with the same number.

DETAILED DESCRIPTION

[011] All scientific and technical terms used herein have meanings commonly used in the art unless otherwise specified. The definitions provided herein are to facilitate understanding of certain terms used frequently herein and are not meant to limit the scope of the present disclosure.

[012] As used in this specification and the appended claims, the singular forms “a”, “an”, and “the” encompass embodiments having plural referents, unless the content clearly dictates otherwise.

[013] As used in this specification and the appended claims, the term “or” is generally employed in its sense including “and/or” unless the content clearly dictates otherwise. The term “and/or” means one or all of the listed elements or a combination of any two or more of the listed elements.

[014] As used herein, “have”, “having”, “include”, “including”, “comprise”, “comprising” or the like are used in their open ended sense, and generally mean “including, but not limited to”. It will be understood that “consisting essentially of”, “consisting of”, and the like are subsumed in “comprising” and the like. For example, a conductive trace that “comprises” silver may be a conductive trace that “consists of” silver or that “consists essentially of” silver.

[015] As used herein, “consisting essentially of,” as it relates to a composition, apparatus, system, method or the like, means that the components of the composition, apparatus, system, method or

the like are limited to the enumerated components and any other components that do not materially affect the basic and novel characteristic(s) of the composition, apparatus, system, method or the like.

[016] The words “preferred” and “preferably” refer to embodiments that may afford certain benefits, under certain circumstances. However, other embodiments may also be preferred, under the same or other circumstances. Furthermore, the recitation of one or more preferred embodiments does not imply that other embodiments are not useful, and is not intended to exclude other embodiments from the scope of the disclosure, including the claims.

[017] Also herein, the recitations of numerical ranges by endpoints include all numbers subsumed within that range (e.g., 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.80, 4, 5, etc. or 10 or less includes 10, 9.4, 7.6, 5, 4.3, 2.9, 1.62, 0.3, etc.). Where a range of values is “up to” a particular value, that value is included within the range.

[018] Use of “first,” “second,” etc. in the description above and the claims that follow is not intended to necessarily indicate that the enumerated number of objects are present. For example, a “second” substrate is merely intended to differentiate from another infusion device (such as a “first” substrate). Use of “first,” “second,” etc. in the description above and the claims that follow is also not necessarily intended to indicate that one comes earlier in time than the other.

[019] Ion-selective electrodes (ISEs) are of great interest in the context of wearable and implantable sensors for continuous online monitoring of physiologically significant ions, such as K^+ , Na^+ , Cl^- , and pH (H^+), as well as other unrelated uses. Potentiometric ion-selective sensing systems consist of a working electrode that responds to the ion of interest and a reference electrode that provides an invariant sample-independent reference potential. To integrate these sensors into

wearable and implantable devices, both electrodes have to be miniaturized, and they must be built from biocompatible materials.

[020] Conventional reference electrodes used in potentiometry, such as the well-known Ag/AgCl electrode, contact the sample through a salt bridge that contains nearly equitransferrant cations and anions to provide a sample-independent reference potential. However, salt bridges have drawbacks, such as the need for maintenance and the mutual contamination of the bridge electrolyte and sample solution. To address these limitations, reference electrodes that comprise a reference membrane doped with ionic liquids or salts of different hydrophilicity have been proposed. These devices are designed so that the ionic liquid or salt slowly leaches out of the reference membrane and, thereby, establishes a sample-independent distribution potential at the interface of the reference membrane and the sample.

[021] The polymeric membrane material most commonly used in such reference electrodes is plasticized poly(vinyl chloride) (PVC), which has also been very popular for the fabrication of ISE membranes. Plasticizers lower the glass transition temperature (T_g) of PVC below room temperature and, thereby, lower the electrical resistance of ion-doped plasticized PVC membranes by many orders of magnitude below that of unplasticized membranes. However, these plasticizers may also gradually leach out of reference and ion-selective membranes, which can be a concern because many of them are known as endocrine disruptors and inducers of inflammatory reactions.

[022] While much effort has been made to replace ISE membranes made of plasticized PVC with plasticizer-free polymers, such as silicones, polyurethanes, polyacrylates, and polymethacrylates, much less attention has been paid to the membrane matrix of ionic liquid based reference electrodes. The use of plasticizer-free polyurethane and poly(*n*-butyl acrylate) for the preparation

of polymeric membrane reference electrodes was reported, but in both cases the membranes were doped with a hydrophobic salt rather than an ionic liquid. To date, the only reference electrodes with a plasticizer-free reference membrane doped with an ionic liquid were fabricated with a poly(vinylidene fluoride-*co*-hexafluoropropylene) matrix.

- [023] Disclosed herein are plasticizer-free silicones as polymer matrixes for reference membranes. Such novel membranes may offer certain advantages: (i) Silicones are widely used in implantable devices, and a range of commercially available silicones have been certified as biocompatible or medical grade following manufacturing standards guided by government agencies, such the US Food and Drug Administration. (ii) Silicones typically have a very low T_g , which is a prerequisite for use in ISE and reference membranes. (iii) Silicone precursors can be easily dissolved and cast into thin films. (iv) The curing chemistry of silicones offers an opportunity to covalently attach reference membranes to a range of substrates with surface hydroxyl groups to prevent membrane delamination.
- [024] Disclosed herein are polymeric reference electrode membranes prepared by solvent casting from seven (7) commercially available biocompatible silicones that are widely used in implantable devices. Reference electrodes with membranes consisting of poly(3,3,3-trifluoropropylmethylsiloxane) (Fluorosilicone 1) and one of several 1-methyl-3-alkylimidazolium bis(trifluoromethylsulfonyl)imide ionic liquids provided a stable and sample-independent potential in electrolyte solutions spanning the range of electrolyte concentrations in human blood, with more hydrophobic ionic liquids performing better. Over 8 days at 37 °C in artificial blood electrolyte solutions, the reference membranes doped with 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide exhibited a potential drift as low as 20 μ V/h. In 10% animal serum, a 112 μ V/h drift was observed over 5.8 days. Also disclosed herein are

silicone materials doped with an ionic liquid that either failed to form self-standing membranes or did not provide a sample-independent potential in the ionic concentration range tested. In case of the functional reference electrodes, differential scanning calorimetry confirmed good miscibility between the ionic liquid and the polymer matrix, whereas the poor miscibility of four polymer matrixes and the ionic liquids----as confirmed by differential scanning calorimetry---- correlated with an undesirable sample dependence of the reference potential.

[025] FIG. 1 is a schematic of an illustrative embodiment of a reference electrode described herein.

The reference electrode 100 includes solid electron conductor 102 at the distal end. Electrode 100 also includes membrane 104 and interlayer 106. Interlayer 106 is disposed between conductor 102 and membrane 104. In some embodiments, solid electron conductor 102 can be a disk, for example, a gold disk. Electrode 100 may also include electrode body 110. Electrode body 110, conductor 102, interlayer 106, membrane 104 may all be housed in an optional housing (not pictured herein) that would be positioned around the electrode body 110. A screw cap would also be included with the optional housing, which when fastened to the housing would bring the components of the electrode into operable contact. Electrode 100 can also include electrode connector 120 connecting conductor 102 to a recording device, processor, or computer that can record the information from the reference electrode 100.

[026] The size and/or shape of the electrodes can vary depending on the specific use and all are within the scope of this invention. The electrodes may be configured for home or point-of-care use, for use at a hospital, clinic, or other similar settings. This may include miniaturization of the electrodes. The electrodes, for example, can be a planar device in which the solid electron conductor, an interlayer comprising mesoporous carbon, and a membrane are applied as

consecutive layers on an inert substrate, such as plastic, silicon, or paper. The electrodes may be paper-based systems. These paper-based systems may be disposable and miniaturized.

[027] The solid electron conductor 102 can include a variety of conducting materials. Illustrative conducting materials include gold, silver, platinum, copper, stainless steel, carbon, indium-tin-oxide (ITO), fluorine-doped tin oxide (FTO), aluminum and the like. In some embodiments, the solid electron conductor 102 can include gold, platinum, copper, stainless steel, carbon, indium-tin-oxide (ITO), fluorine-doped tin oxide (FTO), aluminum and the like. In some embodiments, the solid electron conductor can include gold. In some embodiments, the solid electron conductor can include a gold disk. The thickness and the diameter of the conductor can vary. The gold disk, for example, can have a diameter of between about 0.5 mm and about 5 mm. Diameters outside of this range are also within the scope of this disclosure. In some embodiments, the diameter of the gold disk can be about 2 mm.

[028] Illustrative materials for use in the interlayer 106 can include, for example, carbon, conductive polymers, metal nanoparticles and nanostructures. In some embodiments, the interlayer 106 can include conducting polymers such as poly(3,4-ethylenedioxythiophene) (PEDOT), poly(3-ocylthiophene) (POT), polypyrrole, or polyaniline. In some embodiments, the interlayer 106 can include three-dimensionally ordered microporous (3DOM) carbon, CIM carbon, carbon nanotubes, graphene, fullerene, or porous carbon spheres, gold nanoporous films, gold nanoparticles, platinum nanoparticles supported on carbon black.

[029] The membrane 104 included in the electrode 100 can generally be described as biocompatible polymeric membranes that can more specifically be described as a plasticizer-free silicone-containing polymer matrix that contains an ionic liquid. The polymeric membrane can be formed from a polymer composition that includes at least one silicone-containing polymer composition

and at least one ionic liquid. Both the polymeric membrane and the polymer composition can be described as plasticizer free or essentially plasticizer free. By plasticizer free, it is meant that the polymeric membrane or the polymer composition has less than 5 wt% plasticizer, less than 1 wt% plasticizer, or less than 0.1 wt% plasticizer free. In some embodiments, the amount of plasticizer can be determined by soaking the membrane (e.g., the polymer composition) in an organic solvent in order to leach the plasticizer from the membrane and then analyze the solvent using high pressure liquid chromatography (HPLC). In some embodiments, differential scanning calorimetry (DSC) can be utilized. To calculate wt% from a DSC-determined T_g , the Fox equation is used $1/T_g = w^1/T_g^1 + w^2/T_g^2$.

[030] The silicone-containing polymer composition and the ionic liquid are miscible with each other. Whether or not the silicone-containing polymer and the ionic liquid are miscible with each other can be determined using a thermodynamic method that shows a single glass transition temperature (T_g) of a composition including only the at least one silicone-containing polymer and the at least one ionic liquid containing the silicone-containing polymer. Illustrative thermodynamic methods of determining a single T_g can include differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), thermal mechanical analysis (TMA), dynamic mechanical analysis (DMA), and dielectric thermal analysis (DETA) for example. In some embodiments, DSC can be utilized.

[031] Illustrative ionic liquids can include, for example, those that include an imidazolium cation or quaternary ammonium cation group; and an anion comprising a bis(perfluoroalkylsulfonyl)imide, an alkyl(perfluoroalkyl)sulfonylimide, a bis(alkylsulfonyl)imide, or a bis(alkylsulfonyl)imide with one or more ether, ester or amide groups interrupting the alkyl chain. More specific examples can include, for example, 1-methyl-

3-octylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$), 1-methyl-3-decylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$), 1-methyl-3-dodecylimidazolium dodecylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$), tributylmethylammonium bis(trifluoromethylsulfonyl)imide ($[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$), and combinations thereof.

[032] The amount of the ionic liquid in useful polymer compositions can vary. For example, in some useful polymer compositions, the amount of the ionic liquid can be at least 5 wt% or at least 10 wt% based on the total weight of the polymer composition. For example, in some useful polymer compositions, the amount of the ionic liquid can be not greater than 75 wt%, not greater than 50 wt%, not greater than 40 wt%, or not greater than 30 wt% based on the total weight of the polymer composition.

[033] In some embodiments, useful silicone-containing polymers can have a glass transition temperature (T_g) that is lower than room temperature. In some embodiments, useful silicone-containing polymers can be polysiloxane based polymers. More specific descriptions of useful silicone-containing polymers can include polysiloxane-based fluorine-containing polymers, polysiloxane-based methyl group-containing polymers, polysiloxane-based cyano group-containing polymers, or combinations thereof. In some embodiments, a useful polymer can include, for example, poly([3,3,3-trifluoropropyl]methylsiloxane).

[034] The amount of the silicone-containing polymer in the polymer composition can vary. For example, in some useful polymer compositions, the amount of the silicone-containing polymer can be at least 5 wt%, or at least 10 wt% based on the total weight of the polymer composition. For example, in some useful polymer compositions, the amount of the silicone-containing

polymer can be not greater than 50 wt%, not greater than 40 wt%, or not greater than 30 wt% based on the total weight of the polymer composition.

- [035] The polymer composition can also include optional components such as inorganic fillers, solvents, or hydrophobic salts. Illustrative inorganic fillers can include, for example silica and titanium dioxide. Illustrative solvents can include, for example tetrahydrofuran (THF), dioxane, dichloromethane (DCM), acetone, dimethylformamide (DMF), chloroform, ethyl acetate (EA), cyclohexane, dimethylsulfoxide, or the combination of two or more.
- [036] In order to form a membrane from a polymer composition, various well known methods can be utilized. In some embodiments, useful illustrative methods can include, for example, solvent casting, spin-coating, dip-coating, or inkjet-printing.
- [037] Useful reference electrodes can include those that provide sample-independent and long-term stable electric potential in the range of ion concentrations regularly found in saliva, urine, interstitial fluid, blood, blood serum, or sweat at room or body temperature, for example.
- [038] The present description also includes an electrochemical sensor system or an electrochemical measurement system. The electrochemical sensor system components include reference electrodes as described herein as well as ion sensitive electrodes (ISEs). In some embodiments, the electrochemical system can also include optionally 2, 3 or 4 electrodes, a workstation, a processor, or any combinations thereof. The electrochemical sensor system components may also include a work station, power source, sample holder, computer and/or recorder with optionally a display. The recording device and/or computer can record and/or compute the amount or concentration of an analyte in a sample. The components of the electrochemical sensor systems may be integral within the system, i.e., housed together, or they may be separate components that can be electrically connected to form the electrochemical sensor systems.

[039] The electrochemical sensor system may be configured to be, for example, a potentiometric sensor, an ion-sensitive field effect transistor, a voltammetric sensor, an amperometric sensor, a coulometric sensor or an impedance sensor. A general electrochemical measurement system can include, for example, an electrochemical workstation that can be connected to a plurality of electrodes. The electrochemical workstation may be connected to two, three, or four electrodes. All of the electrodes can be in contact with the sample. In one exemplary embodiment with a potentiometric system, a reference and sensing electrode are connected to the workstation, which is referred to as a potentiometer. In an embodiment with a voltammetric system, a reference, an auxiliary, and a sensing electrode are connected to the workstation. Impedance measurements can be performed with two, three, or four electrodes. Other components known in the art may also be present and may also be part of the electrochemical system, and all are within the scope of the present description.

[040] The present description includes paper-based sensing devices. The paper-based devices can be disposable and may be potentiometric sensing devices. A variety of ions or analytes in samples can be detected in the paper-based devices as described above for the ion-selective electrodes including, for example, H^+ , Li^+ , Na^+ , K^+ , Mg^{2+} , Ca^{2+} , Cu^{2+} , Ag^+ , Zn^{2+} , Cd^{2+} , Hg^{2+} , Pb^{2+} , NH_4^+ , carbonate, bicarbonate, nitrate, nitrite, sulfide, chloride, iodide, and the like, as well as organic anions and cations such as heparin, protamine, and the like. The ISE can be stencil-printed Ag/AgCl electrode.

[041] The present description includes a method for measuring analytes in a sample. The analytes are generally ions in a sample. The ions may be in natural substances or synthetic substances. The samples can be clinical samples, environmental samples, industrial samples, forensic samples, agricultural samples, and the like. Clinical samples can include samples generated in hospitals,

clinics, in point-of-care scenarios such as home or other settings. Clinical samples can be from healthy individuals or individuals with medical issues. The samples may be liquid or gas samples. If the sample is a gas, the electrochemical sensor may be or may not be separated from the sample by a gas-permeable membrane.

[042] Methods of using devices including disclosed reference electrodes, methods of obtaining measurements, other similar methods, or combinations of such methods can be similar to those disclosed in U.S. Patent No. 9,874,539, the disclosure of which is incorporated herein by reference thereto.

[043] The present disclosure is further illustrated by the following examples. It is to be understood that the particular examples, assumptions and procedures are to be interpreted broadly in accordance with the scope and spirit of the disclosure as set forth herein.

[044] **Examples**

[045] **Materials.** Reagents and materials were obtained from the following sources: Dow RTV 730 fluorosilicone (**Fluorosilicone 1**) and RTV 3140 silicone (**Silicone 1**) from Dow Corning (Midland, MI); NuSil silicones MED-1555 (**Fluorosilicone 2**), MED-2000 (**Silicone 2**), MED-6381 (**Silicone 3**), MED-6385 (**Silicone 4**), and GEL-3500 (**Silicone 5**) from NuSil (Carpinteria, CA); high molecular weight PVC and *o*-nitrophenyl octyl ether (*o*-NPOE) from Fluka (Buchs, Switzerland); the ionic liquids 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$), 1-methyl-3-decylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$), 1-methyl-3-dodecylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$), and tributylmethylammonium bis(trifluoromethylsulfonyl)imide ($[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$) from Iolitec (Tuscaloosa, AL); valinomycin from Sigma-Aldrich (St. Louis, MO); sodium tetrakis[3,4-bis-(trifluoromethyl)phenyl]borate (NaTFPB) from Dojindo

(Kumamoto, Japan). Colloid-imprinted mesoporous (CIM) carbon was prepared as previously reported.³⁹ Autonom freeze-dried animal serum was purchased in powder form from Sero (Billingstad, Norway). All chemicals were used as received. Aqueous solutions were prepared from deionized water treated with a Milli-Q Plus water system (Millipore, Bedford, MA) to give a resistivity of 18.2 MΩ/cm.

[046] **Electrode Fabrication.** In the case of one-component silicone materials (**Fluorosilicone 1**, **Silicone 1**, **Fluorosilicone 2**, **Silicone 2**), solutions to prepare reference electrode membranes were prepared by dissolving 200 mg of the silicone material and 50 mg ionic liquid in 1.0 mL tetrahydrofuran (THF) and stirring with a magnetic stir bar. Then the membrane solution was poured into a circular Teflon Petri dish of 25 mm diameter and left to allow for solvent evaporation and silicone curing. In the case of silicone materials with either only a separate catalyst (**Silicone 4**) or both a separate catalyst and a separate crosslinker (**Silicone 3**), a combined amount of 200 mg of the different components in the recommended ratios was used. The base (i.e., the linear polysiloxane) and (if applicable) the crosslinker were dissolved in 1.0 mL THF, followed by stirring, casting of the solution into a Teflon Petri dish of 25 mm diameter, and addition of catalyst into the cast solution right afterwards. In the case of the adjustable two-component **Silicone 5**, a total amount of 200 mg with a 1:1 mass ratio was used. Both components were dissolved in 1.0 mL THF and cast into a Teflon Petri dish of 25 mm diameter. After complete curing of the silicones, small circular disks (7 mm diameter) were cut from the master membranes and mounted into Philips type electrode bodies⁴⁰ (Glasbläserei Möller, Zürich, Switzerland; Ag/AgCl internal reference) with a 1.0 mM KCl inner filling solution. The electrodes were conditioned in 1.0 mM KCl for two days prior to use.

[047] For the preparation of solid-contact reference electrodes, gold electrodes (planar circular Au electrodes with a 2 mm diameter, embedded into an inert Kel-F polymer shaft; CH Instruments, Austin, TX) were polished on a polishing cloth, first with 0.5 μm and then with 0.03 μm aqueous aluminum oxide slurry (Buehler, Lake Bluff, IL), and then sonicated first in water and then in ethanol, each for 6 min. A stream of argon was used to dry the electrodes. A CIM carbon suspension solution was prepared by dissolving 47.5 mg CIM carbon and 2.5 mg **Fluorosilicone 1** in 1.0 mL THF followed by sonication for 30 min. Then, 30 μL of this suspension was dropcast onto the gold electrode and left to dry. This was followed by two aliquots (20 μL followed by 30 μL) of silicone membrane solution dropcast onto the CIM carbon layer. After complete curing of the silicones, the electrodes were conditioned for two days in 1.0 mM KCl solution prior to use. A water layer test using K^+ -selective **Fluorosilicone 1** solid-contact electrodes showed no undesired water layer between **Fluorosilicone 1** and the solid contact material.

[048] Solutions for the preparation of ionic liquid-doped PVC membranes were prepared by dissolving 100 mg high molecular weight PVC, 100 mg of the plasticizer *o*-NPOE, and 50 mg $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ in 1.0 mL THF. Solutions for the fabrication of blank plasticized PVC membranes were prepared by dissolving 100 mg high molecular weight PVC and 100 mg of the plasticizer *o*-NPOE in 1.0 mL THF. In both cases, the solutions were stirred until the PVC had completely dissolved and then poured into a glass Petri dish of 22 mm diameter and left to dry over 24 h to give a plasticized PVC membrane.

[049] **Electrochemical Measurement.** Potentiometric measurements were performed with a 16-channel potentiometer (Lawson Labs, Malvern, PA) controlled by EMF Suite software (Fluorous Innovation, Arden Hills, MN). Potential responses of all polymeric membrane reference

electrodes were measured in stirred solutions against a conventional free-flow double-junction reference electrode (DX200, Mettler Toledo, Switzerland; AgCl saturated 3.0 M KCl as inner reference electrolyte and 1.0 M LiOAc as bridge electrolyte). Unless mentioned otherwise, all measurements were performed at room temperature (20 °C). For KCl calibrations, measurements began at 1.0 mM KCl, and aliquots of concentrated KCl solutions were added to double the concentration stepwise to 16 mM. All emf values were corrected for the liquid junction potentials of the double-junction reference electrode. Activity coefficients were calculated based on a two parameter Debye-Hückel approximation. Membrane resistances were measured with the known shunt method.

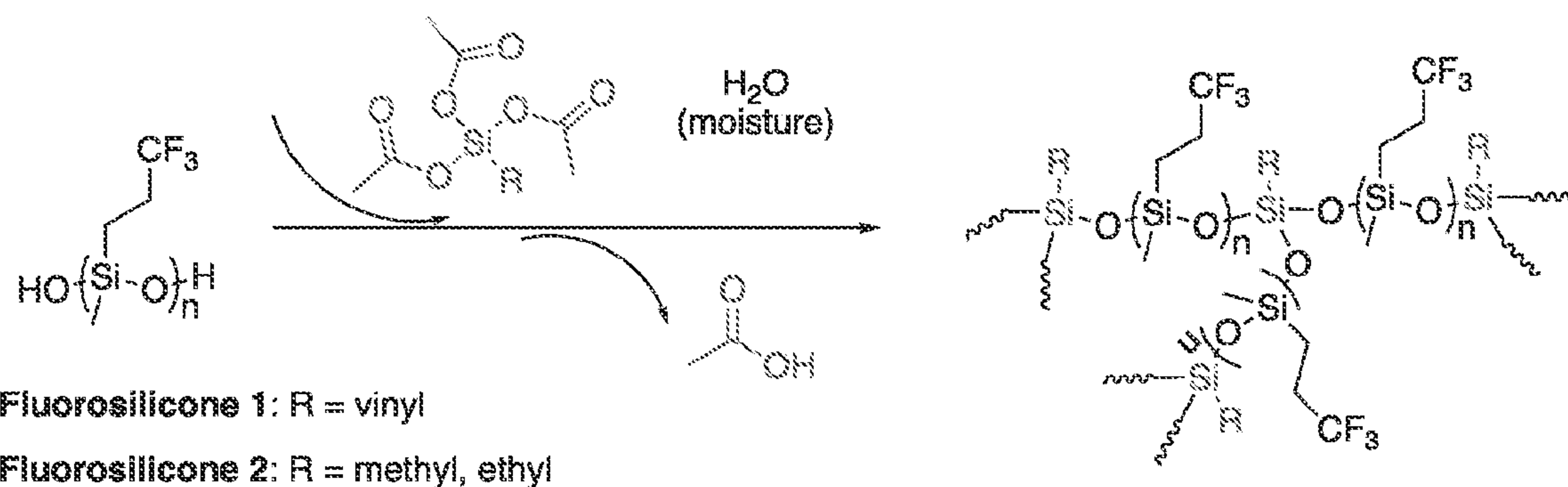
[050] **Differential Scanning Calorimetry.** Cast membranes were left to dry for at least two weeks prior to DSC experiments to ensure that the membranes were fully cured, and all solvent had evaporated. Experiments were performed with a TA Instrument Q1000 DSC (New Castle, DE) with a liquid nitrogen cooling system. Small pieces of samples were cut from the master membranes and enclosed in a lidded sample pan. They were first thermally equilibrated at 40 °C for one minute. Then the samples were scanned to -180 °C and back to 40 °C, with a consistent 1.0 °C/min heating and cooling rate. Glass transition temperatures were determined as midpoints of transition zones.

[051] **Results and Discussion**

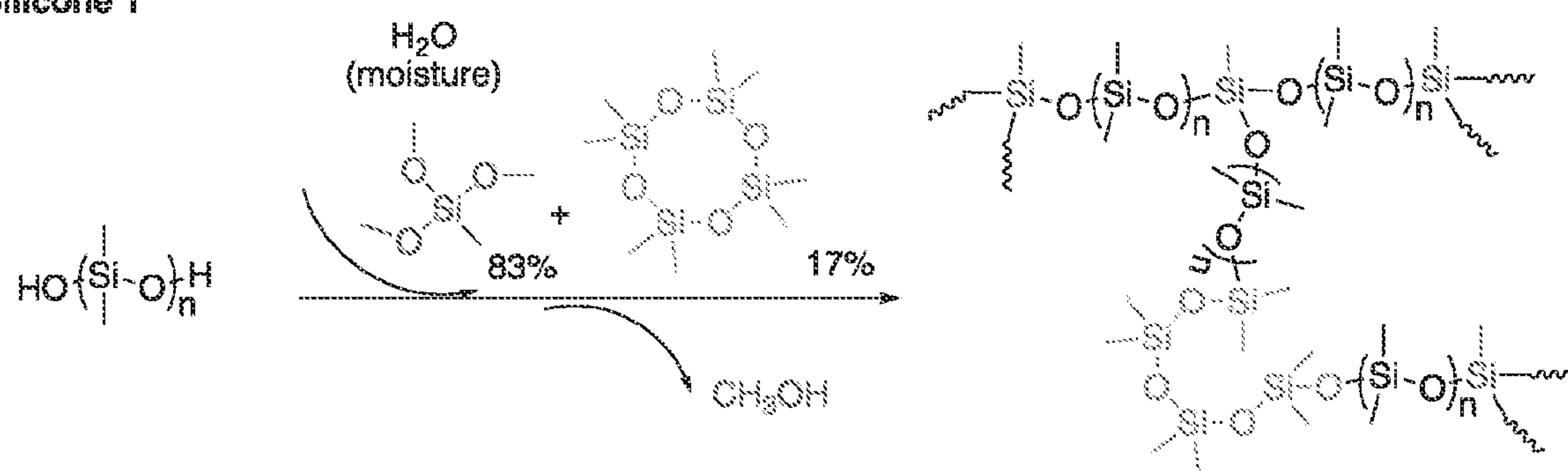
[052] **Silicone Materials and Sensor Membrane Fabrication.** Seven commercially available silicones were used in this work. **Fluorosilicone 1**, **Fluorosilicone 2**, **Silicone 1**, and **Silicone 2** are so-called one-component silicones. Once exposed to air, they cure upon reaction with water from the atmosphere, releasing either an alcohol or acetic acid as byproduct. Typically, they contain an organotin catalyst to facilitate this reaction (see Scheme 1). The three other silicone

materials used in this work were prepared by mixing multiple components. **Silicone 4** is prepared from two components. One of these components contains a tin catalyst and is used in a few weight percent with respect to the other component, which contains the linear polysiloxane backbone and crosslinker. In the case of **Silicone 3**, the crosslinker is provided separately as a third component. In the case of **Silicone 5**, the two components are added in a 1:1 ratio. Curing occurs upon mixing of the two or three components in a ratio recommended by the manufacturer (see Electrode Fabrication). From a fabrication point of view, one-component silicones are advantageous as their use reduces the number of fabrication steps and eliminates the need to control the ratio of multiple components.

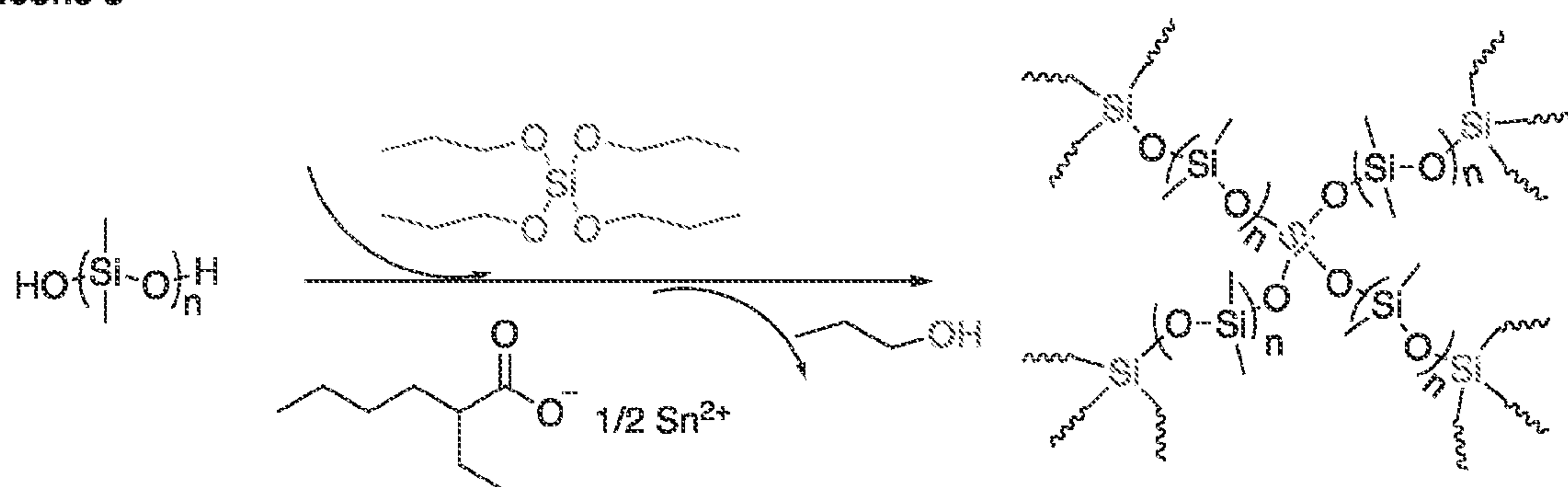
Fluorosilicone 1 and Fluorosilicone 2



Silicone 1



Silicone 3



Scheme 1. Curing chemistries of representative fluorosilicones and silicones studied in this work (see also Table 1).

[053] Even though the exact structures of these silicones differ from each other in a number of ways, their linear backbones are either poly(3,3,3-trifluoropropylmethylsiloxane) (**Fluorosilicone 1** and **Fluorosilicone 2**, referred to in the following as fluorosilicones) or poly(dimethylsiloxane) (**Silicone 1**, **Silicone 2**, **Silicone 3**, **Silicone 4**, and **Silicone 5**). Many silicone materials also

contain fillers such as silica or titanium dioxide to improve their mechanical properties (see Table 1). Some of these fillers can be removed by centrifugation if that is desired for sensing purposes.

[054] The electrical resistances of blank silicone membranes that were not doped with an ionic liquid all fall into the relative narrow range of 0.2 to 2.0 GΩ, except for **Silicone 1** (15 GΩ). These resistances are very high, but they are not infinite as it would be expected for an ideal material without ionic impurities. This suggests that the different silicone materials have low levels of intrinsic ionic impurities. Notably, there is no evident correlation between membrane resistances and the type of silicone.

[055] Casting of **Silicone 3** gave self-standing membranes in both its pure form and when doped with 20 wt % [C₈mim⁺][NTf₂⁻]. However, when **Silicone 3** was doped with 20 wt % [C₈mim⁺][NTf₂⁻], clear liquid aggregates with average sizes of 1 to 2 mm diameter macro-phase separated from the polymer, as could be readily detected with the naked eye. In three attempts to cast such membranes, varying patterns and shapes of liquid aggregates appeared. Areas were randomly selected for cutting out smaller circular disks for use in electrodes. Casting of the two-component **Silicone 4** and **Silicone 5** did not give self-standing membranes, independently of whether it was in a pure form or doped with 20 wt % [C₈mim⁺][NTf₂⁻].

[056] Table 1. Characteristics of Reference Electrode Membranes.

Membrane matrix	Silicone type	Polymer backbone	Biocompatibility	Resistance (doped with [Csmim ⁺][NTf ₂ ⁻]) /MΩ ^a	Fillers
Fluoro-silicone 1	1 component, acetic acid evolving	poly[(3,3,3-trifluoropropyl)methylsiloxane], OH-terminated ^b	no plasticizer	4.7 ± 4.0	titanium dioxide, silica
Fluoro-silicone 2	1 component, acetic acid evolving	poly[(3,3,3-trifluoropropyl)methylsiloxane], OH-terminated	medical grade ^c	8.0 ± 2.7	silica
Silicone 1	1 component, alcohol evolving	poly(dimethylsiloxane), OH-terminated	no plasticizer	(7.1±7.9)×10 ³	silica
Silicone 2	1 component, acetic acid evolving	n.a. ^d	medical grade ^c	(4.9±6.5)×10 ³	silica
Silicone 3^e	3 components (base, cross-linker, Sn catalyst)	poly(dimethylsiloxane), OH-terminated	medical grade ^c	160 ± 120	none
Silicone 4^f	2 components (base, Sn catalyst)	n.a. ^d	medical grade ^c	n.a.	silica
Silicone 5^g	2 components, Pt catalyst	poly[dimethylsiloxane-co-(3,3,3-trifluoropropyl)methylsiloxane], vinyl-terminated	no plasticizer	n.a.	none
Plasticized PVC	n.a.	poly(vinyl chloride)	plasticizer required	0.59 ± 0.08 ^h	none

^aElectrical resistance of membranes doped with 20 wt % [Csmim⁺][NTf₂⁻] (n = 4). ^bNumerical

locants of fluorines inferred from Chemical Abstract number; material data safety sheet only

specifies trifluoropropyl. ^cMedical grade for intended use in, on, or in contact with the body,

according to regulatory standards: Current Good Manufacturing Practice 21 Code of Federal

Regulations § 820 (Device) and § 210-211 (Drug/Active Pharmaceutical Ingredient) from the

US Food and Drug Administration and ISO 9001 from the International Organization for Standardization.⁴⁵ ^d Exact structure not available from manufacturer. ^e Membranes doped with 20 wt % [C₈mim⁺][NTf₂⁻] contained liquid aggregates. ^f **Silicone 4** Membrane does not form robust self-standing membranes. ^g At the 1:1 component ratio recommended by the manufacturer, **Silicone 5** does not form robust self-standing membranes. ^h n = 4.

[057] **Responses to KCl of Reference Electrode Membranes Doped with [C₈mim⁺][NTf₂⁻].** The response mechanism of reference electrodes with ionic liquid-doped reference membranes relies on the control of the phase boundary potential at the membrane–sample interface by distribution of the ionic liquid between the two phases. Sample ions most likely to negatively affect the reference potential have a comparatively small hydration energy, which lowers the energy barrier for transfer into the reference membrane. Considering physiological ion concentrations in human blood serum and typical ion transfer energies, as apparent, e.g., from the selectivity of ionophore-free ion exchanger ISEs, one of the ions of most concern is potassium, which has a mean concentration of 4.10 mM in human blood serum of healthy adults (95% range: 3.5 to 4.7 mM).

[058] In order to assess whether potassium affects the ideally sample-independent response of reference electrodes with ionic liquid doped silicone membranes, the use of these electrodes was tested in the KCl concentration range from 1.0 to 16 mM. When doped with [C₈mim⁺][NTf₂⁻], only reference electrode membranes based on the **Fluorosilicone 1** matrix provided a sample-independent potential. The other silicone materials (**Silicone 1**, **Fluorosilicone 2**, **Silicone 2**, and **Silicone 3**) did not give reference potentials independent of the KCl concentration. Given the similar polarities of all of these silicone and fluorosilicone matrixes, it appears unlikely that differences in the free energies of transfer of K⁺ or Cl⁻ from aqueous samples into the reference

membranes explain these results alone. Indeed, the electrical resistance of the silicones doped with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ and the DSC results suggest that the poor miscibility between the ionic liquid and the silicone polymer is the major cause for the poor performance as reference electrode membranes.

[059] **Functionality of Reference Electrodes and Miscibility of Ionic Liquids with Silicone Materials.** Because **Fluorosilicone 1** doped with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ emerged as the most successful matrix for reference electrodes, we used DSC to further characterize this material. For comparison, we also used DSC to test the pure ionic liquid as well as undoped and $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -doped membranes with polymer matrixes consisting of **Fluorosilicone 2**, **Silicone 1**, **Silicone 2**, or **Silicone 3**. Table 2 summarizes the glass transition temperatures determined from the DSC traces.

[060] **Table 2. Glass Transition Temperatures of the Ionic Liquid $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$, Neat Polymers, and $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -Doped Polymers.**

Material	Undoped (°C)	Doped with 10 wt % $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ (°C)	Doped with 20 wt % $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ ^b (°C)
$[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$	-84 ^a	n.a.	n.a.
Fluorosilicone 1	-67	-66	-66 and -83 ^b
Fluorosilicone 2	-67	-67 and -83 ^b	-67 and -82 ^b
Silicone 1	-117	-117 and -84 ^b	-116 and -84 ^b
Silicone 2	-120	-120 and -82 ^b	-119 and -83 ^b
Silicone 3	-117	-118 and -83 ^b	-121 and -81 ^b
Plasticized PVC	-54	n.a.	-56

^a T_g of pure $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$. ^bTwo glass transitions observed, one corresponding to pure $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$.

[061] At 10 wt % doping with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$, **Fluorosilicone 1** only shows one T_g at -66 °C, which coincides within error with the T_g of undoped **Fluorosilicone 1** at -67 °C. This indicates that at

this doping level, the ionic liquid and **Fluorosilicone 1** are fully miscible. When the doping level is increased to 20 wt %, an additional T_g consistent with the ionic liquid appears, which is a clear sign for phase separation. This confirms that the miscibility of $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ in **Fluorosilicone 1** is at least 10 wt % and is consistent with the electrical resistance of these membranes, which is two orders of magnitude lower than for undoped **Fluorosilicone 1**.

[062] For all other fluorosilicone and silicone matrixes studied by DSC, even at the low doping level of 10 wt % the DSC trace shows two glass transitions, one corresponding to the T_g of the pure ionic liquid, and the other corresponding to the T_g of the undoped polymer, indicating phase separation. Moreover, addition of $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ to these membrane matrixes has within error no effect on the membrane resistance, except for the case of **Fluorosilicone 2**. Although $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ and **Fluorosilicone 2** are not fully miscible at the 10 wt % doping level, the amount of dissolved $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ was sufficient to lower the membrane resistance to some extent. All of this is consistent with a poor miscibility between $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ and these polymers and the poor performance of these matrixes as reference membranes.

[063] For comparison, DSC tests were also performed with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -doped PVC membranes plasticized with *o*-NPOE. At the doping level of 20 wt %, which is the weight ratio that was used in prior work,²³ the plasticized PVC membranes exhibited only one T_g . Moreover, doping of the plasticized PVC membrane with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ lowered the membrane resistance to 0.59 M Ω , consistent with a high solubility of the ionic liquid in the plasticized PVC matrix.

[064] Based on these DSC and resistance data, we conclude that the poor performance of many of the **Fluorosilicone 2**, **Silicone 1**, **Silicone 2**, and **Silicone 3** membranes doped with ionic liquid is primarily explained by the low solubility of the ionic liquid in the polymer matrix, which results

in a high electrical resistance but may also affect the amount of ionic liquid distributed across the membrane/sample phase boundary.

[065] **Effect of Sample Concentration on Solid-Contact Reference Electrodes with Fluorosilicone 1 Membranes.** In view of the results obtained with conventional electrode bodies with an inner filling solution as discussed above, only **Fluorosilicone 1** was used for subsequent work with solid-contact reference electrodes. CIM carbon, a hydrophobic, highly pure carbon material with a high surface area ($321 \text{ m}^2/\text{g}$) and a low surface concentration of polar functional groups (0.053 mmol/m^2 ketone groups; phenol, lactone, lactol, and carboxylic acid group concentrations too low to detect) was used as transducer layer between the reference membrane and gold as the underlying electron-conducting metal. In the 1.0 mM to 16 mM KCl range, solid-contact **Fluorosilicone 1** reference electrodes showed a sample-independent emf response, with an emf dependence on the logarithm of the K^+ activity of $0.0 \pm 0.7 \text{ mV/decade}$ ($n = 3$). The difference between the maximum and minimum potentials over the entire activity range was $2.0 \pm 1.4 \text{ mV}$ ($n = 3$). This demonstrates that the good characteristics of **Fluorosilicone 1** reference membranes carry over to the solid-contact setup with CIM carbon as transducer.

[066] To simulate the working environment of wearable or implantable sensors, electrolyte solutions mimicking critical blood parameters in terms of pH and K^+ , Na^+ , Cl^- , and phosphate concentrations were used. Specifically, two such electrolyte solutions, designated below as HIGH and LOW, were prepared (see Table 3 for their composition) to represent unusually high and low blood electrolyte concentrations. Figure 3 confirms that **Fluorosilicone 1** reference electrodes provide the same potential in HIGH and LOW solutions.

[067] **Table 3. Composition of Artificial Blood Electrolyte Solutions and Potential Stability Characteristics of Reference Electrodes with [C₈mim⁺][NTf₂⁻]-doped Fluorosilicone 1 Membranes Tested in Artificial Blood Electrolyte Solutions.**

Electrolyte	HIGH (mM)	LOW (mM)
KCl	4.7	3.5
NaCl	102.8	96.4
Na ₂ HPO ₄	0.82	0.74
NaH ₂ PO ₄	0.18	0.26
pH	7.45	7.35

[068] **Long-term Stability in Artificial Blood Electrolyte Solution.** To test the long-term stability of reference electrodes with **Fluorosilicone 1** membranes, their emf was tested in a 1:1 mixture of HIGH and LOW solution (see Table 3) in a temperature-controlled box at 37 °C. The drift over 8 days was only $-88 \pm 59 \mu\text{V/h}$ ($n = 3$), with the best performing electrode giving a drift of only 20 $\mu\text{V/h}$ (see Figure 4). The reference electrodes were also tested for long-term stability in tenfold diluted animal serum solution (37 °C). Over 5.8 days, a drift of $112 \pm 26 \mu\text{V/h}$ ($n = 3$) was observed, which is within error the same as for measurements in artificial electrolyte solutions. For comparison, **Fluorosilicone 1** reference membranes doped with [C₈mim⁺][NTf₂⁻] tested with a conventional electrode setup with an inner filling solution gave a drift of $27 \pm 14 \mu\text{V/h}$ ($n = 3$) over 3 days in artificial blood electrolyte solution. This suggests that additional improvements in the fabrication may further reduce the drift of the solid-contact reference electrodes.

[069] **Comparison of Reference Membranes doped with Different Ionic Liquids.** In view of possible further improvements in potential stability, we carried out experiments with **Fluorosilicone 1** reference membranes doped with several other ionic liquids. Key requirements to make ionic liquids suitable for use in reference electrodes membranes are a moderate hydrophobicity and chemical stability of both the cation and anion.⁴⁷ To this end, three other

ionic liquids were chosen for further work: $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$ and $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$ differ from $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ only in the length of the alkyl chain of the cation, and $[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$ shares the same anion with the other ionic liquids but has a tetraalkylammonium instead of an imidazolium cation. To test these **Fluorosilicone 1** reference electrodes, their potential was again determined in the 1.0 to 16 mM KCl range. EMF results are shown in Figure 5 and potential stability characteristics are summarized in Table 4.

[070] **Table 4. Effect of KCl on EMF of Reference Electrodes with Fluorosilicone 1 Membranes Doped with Different Ionic Liquids.**

Ionic liquid	Slope of linear regression (mV/decade) ^a	Standard ion-transfer potential, $\Delta_{\text{DCE}}^{\text{W}}\phi_i^\circ$ (V) ^b	Potential change over the physiological K^+ range (mV) ^c
$[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$	2.1 ± 0.4	-0.105	0.27
$[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$	-1.5 ± 0.7	-0.113	0.19
$[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$	-0.4 ± 0.6	-0.190	0.05
$[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$	0.2 ± 0.3	-0.220	0.03

^a Average and standard deviation of the slope of emf responses to KCl in the 1.0 to 16 mM activity range ($n = 4$, except for $n = 3$ for $[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$). ^b Experimental standard potential for ion transfer from water to 1,2-dichloroethane. ^c Physiological K^+ range: 3.5 to 4.7 mM.

[071] Over the entire tested range of KCl concentrations, the effect of KCl on the reference potential provided by the three types of reference membranes doped with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$, $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$, and $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$ was minimal. It may appear that $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$, which has the longest alkyl chain and is the most hydrophobic of these three ionic liquids, afforded the smallest variability of the reference potential, but superiority of the $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$ -based over the $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$ -based reference electrode cannot be confirmed with statistical significance.

[072] **Fluorosilicone 1** reference membranes doped with $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$ and $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$ were also tested over five days for their long-term stability. Similar drifts as for the membranes doped with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ were observed, showing here too the differences among the $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$ -, $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$ -, and $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$ -based reference electrodes to be minimal. This is consistent with the conclusion that the long-term stability may have been less influenced by the ionic liquid but more by other factors, such as the variability of the solid contact, minor fluctuations of the half-cell potential of the free-flow double-junction reference electrode with respect to which these new reference electrodes were tested, or variability in sample flow conditions.

[073] While medical grade silicones are used in many medical devices, the ionic liquid too must be considered when assessing the biocompatibility of membrane-based reference electrodes. Unfortunately, toxicology data for ionic liquids are still few, and most available studies focused on cytotoxicity. In one of the few investigations of the toxicity of ionic liquids for mammals, an acute oral median lethal dose (LD_{50}) of $3150 \mu\text{mol}/\text{kg}$ body weight was reported for 1-butyl-3-methylimidazolium chloride in female rats. Considering the typical amount of membrane material used in a reference membrane (e. g., 12 mg silicone polymer and 3 mg ionic liquid) and assuming a worst case scenario in which the ionic liquid of an individual sensor leaches out completely over a short period, a concentration of the ionic liquid of $0.08 \mu\text{mol}/\text{kg}$ body weight would be obtained for a human exposed to this sensor.

[074] **Conclusions**

[075] Reference electrodes fabricated from biocompatible materials and used in wearable and implantable sensors may serve an important role in clinical diagnostics and health monitoring of the future. This work demonstrates that silicones are suitable matrixes for ionic liquid-doped

reference membranes. Silicones are particularly promising for this application because they do not require a plasticizer and are available in a range of biocompatible, commercially available varieties. Use of **Fluorosilicone 1** doped with $[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$, $[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$, or $[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$ provides stable and sample-independent potentials in solutions that contain electrolytes in concentrations representative for a wide range of blood samples. In long-term stability tests, the electrodes exhibited potential drifts as low as $20 \mu\text{V/h}$ over 8 days in artificial blood electrolyte solution and $112 \mu\text{V/h}$ over 5.8 days in 10% animal serum.

[076] **Illustrative Embodiments**

[077] Illustrative embodiments include Embodiment 1 that includes a reference electrode including a solid electron conductor; an interlayer; and a polymeric membrane layer comprising a polymer composition, the polymer composition comprising at least one silicone-containing polymer and at least one ionic liquid, wherein the polymeric membrane layer and the polymer composition are essentially plasticizer free and the silicone-containing polymer and the ionic liquid are miscible as determined using a thermodynamic method that shows a single glass transition temperature (T_g) of a composition including the at least one silicone-containing polymer and the at least one ionic liquid, wherein the interlayer is disposed between the solid electron conductor and the polymeric membrane layer.

[078] Embodiment 2 demonstrates additional illustrative embodiments that include Embodiment 1, wherein the ionic liquid comprises at least one imidazolium cation or quaternary ammonium cation group; and an anion comprising a bis(perfluoroalkylsulfonyl)imide, an alkyl(perfluoroalkyl)sulfonylimide, a bis(alkylsulfonyl)imide, or a bis(alkylsulfonyl)imide anion with one or more ether, ester or amide groups interrupting the alkyl chain.

- [079] Embodiment 3 demonstrates additional illustrative embodiments that include Embodiment 1 or 2, wherein the ionic liquid is selected from 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_8\text{mim}^+][\text{NTf}_2^-]$), 1-methyl-3-decylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_{10}\text{mim}^+][\text{NTf}_2^-]$), 1-methyl-3-dodecylimidazolium bis(trifluoromethylsulfonyl)imide ($[\text{C}_{12}\text{mim}^+][\text{NTf}_2^-]$), tributylmethylammonium bis(trifluoromethylsulfonyl)imide ($[\text{NBu}_3\text{Me}^+][\text{NTf}_2^-]$), and combinations thereof.
- [080] Embodiment 4 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 3, wherein the polymer composition has from 5 to 75 wt% of the ionic liquid based on the total weight of the polymer composition.
- [081] Embodiment 5 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 3, wherein the polymer composition has from 10 to 40 wt% of the ionic liquid based on the total weight of the polymer composition.
- [082] Embodiment 6 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 3, wherein the polymer composition has from 10 to 30 wt % of the ionic liquid based on the total weight of the polymer composition.
- [083] Embodiment 7 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 3, wherein the at least one silicone-containing polymer has a glass transition temperature lower than room temperature.
- [084] Embodiment 8 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 7, wherein the at least one silicone-containing polymer is polysiloxane-based.
- [085] Embodiment 9 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 8, wherein the at least one silicone-containing polymer is a polysiloxane-

based fluorine-containing polymer, a polysiloxane-based methyl group-containing polymer, a polysiloxane-based cyano group-containing polymer, or a combination thereof.

- [086] Embodiment 10 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 9, wherein the at least one silicone-containing polymer is poly([3,3,3-trifluoropropyl]methylsiloxane).
- [087] Embodiment 11 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 10, wherein the polymer composition has from 5 to 50 wt% of the polymer based on the total weight of the polymer composition.
- [088] Embodiment 12 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 10, wherein the polymer composition has from 10 to 40 wt% of the polymer based on the total weight of the polymer composition.
- [089] Embodiment 13 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 10, wherein the polymer composition has from 10 to 30 wt% of the polymer based on the total weight of the polymer composition.
- [090] Embodiment 14 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 13, wherein the polymer composition further comprises at least one kind of inorganic filler.
- [091] Embodiment 15 demonstrates additional illustrative embodiments that include Embodiment 14, wherein the at least one kind of inorganic filler is selected from a group consisting of silica and titanium dioxide.
- [092] Embodiment 16 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 15, wherein the thermodynamic method to determine the T_g is selected from differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron

microscopy (SEM), thermal mechanical analysis (TMA), dynamic mechanical analysis (DMA), and dielectric thermal analysis (DETA).

- [093] Embodiment 17 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 15, wherein the thermodynamic method to determine the T_g is differential scanning calorimetry (DSC).
- [094] Embodiment 18 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 17, wherein the reference membrane is solvent cast, spin-coated, dip-coated, or inkjet-printed from the polymer composition.
- [095] Embodiment 19 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 18, wherein the solid electron conductor comprises silver, gold, platinum, copper, stainless steel, carbon, indium-tin-oxide (ITO), fluorine-doped tin oxide (FTO) or aluminum.
- [096] Embodiment 20 demonstrates additional illustrative embodiments that include any of Embodiments 1 to 19, wherein the reference electrode provides sample-independent and long-term stable electric potential in the range of ion concentrations regularly found in saliva, urine, interstitial fluid, blood, blood serum, or sweat at room or body temperature.
- [097] Embodiment 21 includes an electrochemical measurement system using the reference electrode described in any of Embodiments 1 to 20.
- [098] One skilled in the art will appreciate that the methods and compositions described herein can be practiced with embodiments other than those disclosed. The disclosed embodiments are presented for purposes of illustration and not limitation. One will also understand that components of the methods depicted and described with regard to the figures and embodiments herein may be interchangeable.

Claims

1. A reference electrode comprising:

a solid electron conductor;

an interlayer; and

a polymeric membrane layer comprising a polymer composition, the polymer composition comprising at least one silicone-containing polymer and at least one ionic liquid, wherein the polymeric membrane layer and the polymer composition are essentially plasticizer free and the silicone-containing polymer and the ionic liquid are miscible as determined using a thermodynamic method that shows a single glass transition temperature (T_g) of a composition including the at least one silicone-containing polymer and the at least one ionic liquid, wherein the interlayer is disposed between the solid electron conductor and the polymeric membrane layer.
2. The reference electrode according to claim 1, wherein the ionic liquid comprises at least one imidazolium cation or quaternary ammonium cation group; and an anion comprising a bis(perfluoroalkylsulfonyl)imide, an alkyl(perfluoroalkyl)sulfonylimide, a bis(alkylsulfonyl)imide, or a bis(alkylsulfonyl)imide anion with one or more ether, ester or amide groups interrupting the alkyl chain.
3. The reference electrode according to claim 1, wherein the ionic liquid is selected from 1-methyl-3-octylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_8mim^+][NTf_2^-]$), 1-methyl-3-decylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_{10}mim^+][NTf_2^-]$), 1-methyl-3-dodecylimidazolium bis(trifluoromethylsulfonyl)imide ($[C_{12}mim^+][NTf_2^-]$), tributylmethylammonium bis(trifluoromethylsulfonyl)imide ($[NBu_3Me^+][NTf_2^-]$), and combinations thereof.
4. The reference electrode according to any of claim 1, wherein the polymer composition has from 5 to 75 wt% of the ionic liquid based on the total weight of the polymer composition.
5. The reference electrode according to any of claim 1, wherein the polymer composition has from 10 to 40 wt% of the ionic liquid based on the total weight of the polymer composition.

6. The reference electrode according to any of claim 1, wherein the polymer composition has from 10 to 30 wt % of the ionic liquid based on the total weight of the polymer composition.
7. The reference electrode according to any of claims 1 to 6, wherein the at least one silicone-containing polymer has a glass transition temperature lower than room temperature.
8. The reference electrode according to any of claims 1 to 6, wherein the at least one silicone-containing polymer is polysiloxane-based.
9. The reference electrode according to any of claims 1 to 6, wherein the at least one silicone-containing polymer is a polysiloxane-based fluorine-containing polymer, a polysiloxane-based methyl group-containing polymer, a polysiloxane-based cyano group-containing polymer, or a combination thereof.
10. The reference electrode according to any of claims 1 to 6, wherein the at least one silicone-containing polymer is poly([3,3,3-trifluoropropyl]methylsiloxane).
11. The reference electrode according to claim 1, wherein the polymer composition has from 5 to 50 wt% of the polymer based on the total weight of the polymer composition.
12. The reference electrode according to claim 1, wherein the polymer composition has from 10 to 40 wt% of the polymer based on the total weight of the polymer composition.
13. The reference electrode according to any of claims 1 to 6, or 12, wherein the polymer composition has from 10 to 30 wt% of the polymer based on the total weight of the polymer composition.
14. The reference electrode according to any of claims 1 to 6 or 12, wherein the polymer composition further comprises at least one kind of inorganic filler.
15. The reference electrode according to claim 14, wherein the at least one kind of inorganic filler is selected from a group consisting of silica and titanium dioxide.
16. The reference electrode according to claim 1, wherein the thermodynamic method to determine the T_g is selected from differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), scanning electron microscopy (SEM), thermal mechanical analysis (TMA), dynamic mechanical analysis (DMA), and dielectric thermal analysis (DETA).

17. The reference electrode according to claim 1, wherein the thermodynamic method to determine the T_g is differential scanning calorimetry (DSC).
18. The reference electrode according to any of claims 1 to 6, 12, or 15 to 17, wherein the reference membrane is solvent cast, spin-coated, dip-coated, or inkjet-printed from the polymer composition.
19. The reference electrode according to claim 1, wherein the solid electron conductor comprises silver, gold, platinum, copper, stainless steel, carbon, indium-tin-oxide (ITO), fluorine-doped tin oxide (FTO) or aluminum.
20. The reference electrode according to claim 1, wherein the reference electrode provides sample-independent and long-term stable electric potential in the range of ion concentrations regularly found in saliva, urine, interstitial fluid, blood, blood serum, or sweat at room or body temperature.
21. An electrochemical measurement system using the reference electrode described in any of claims 1 to 6, 12, 15 to 17, 19, or 20.

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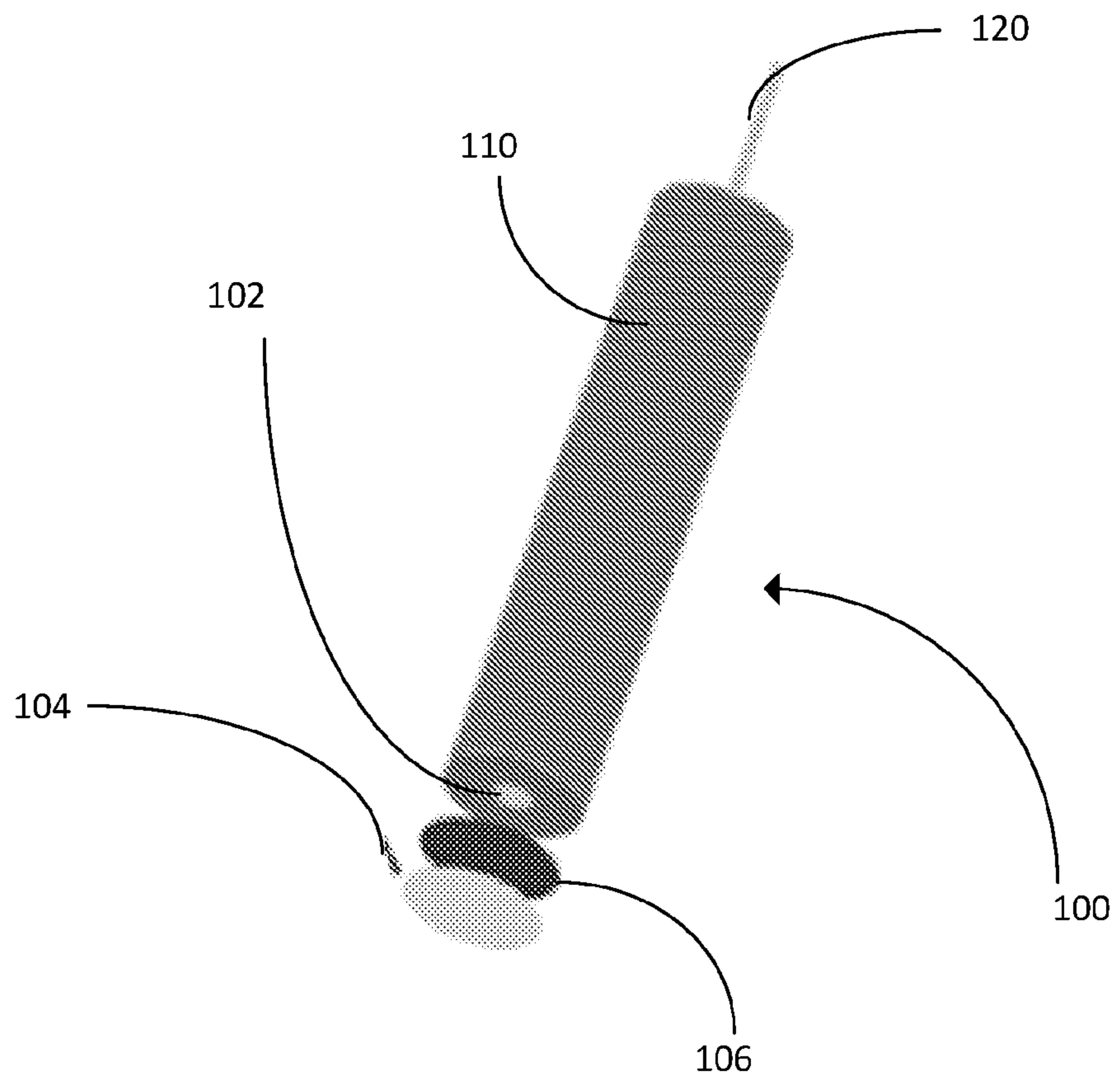


Figure 1

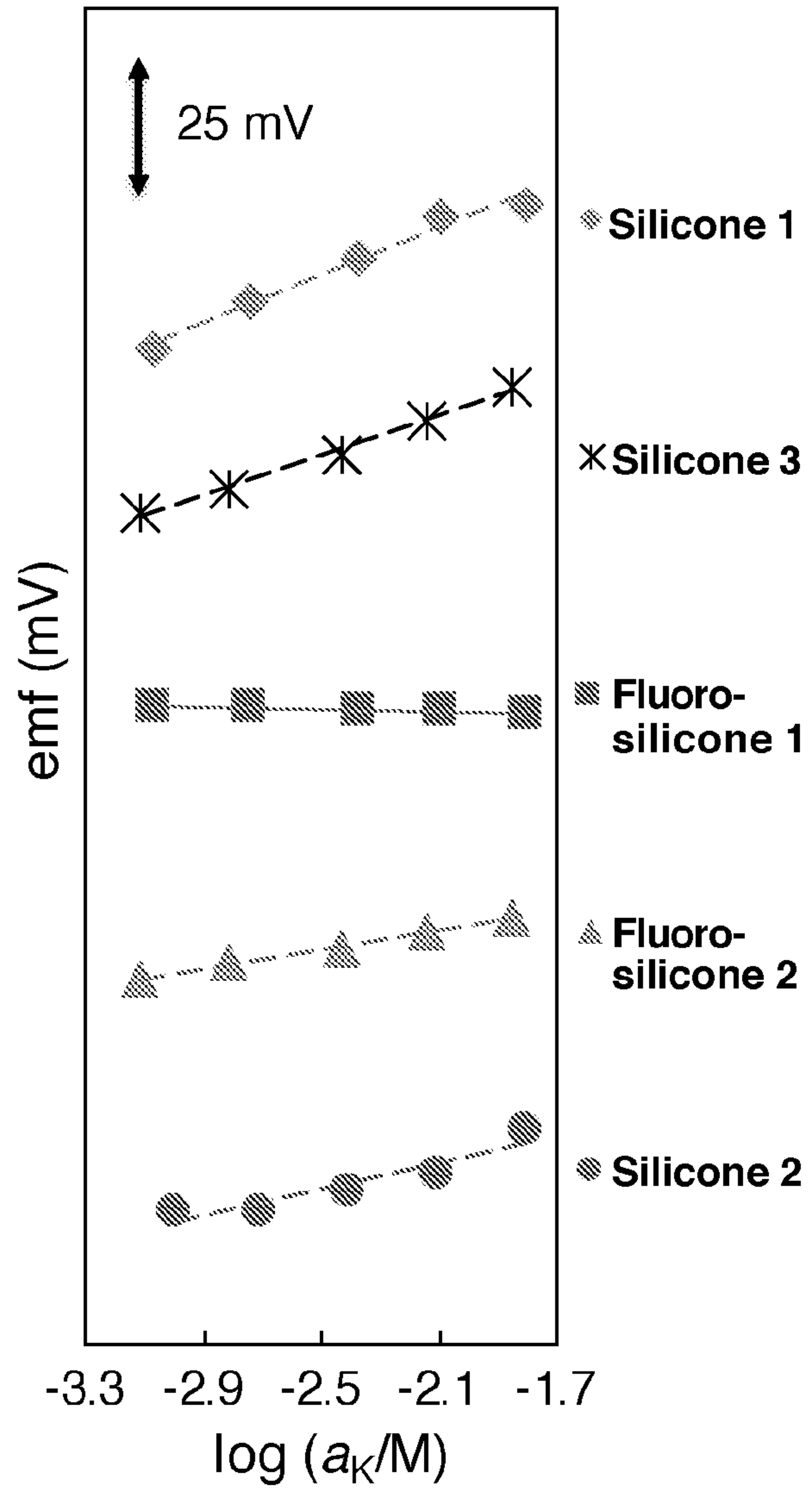


Figure 2

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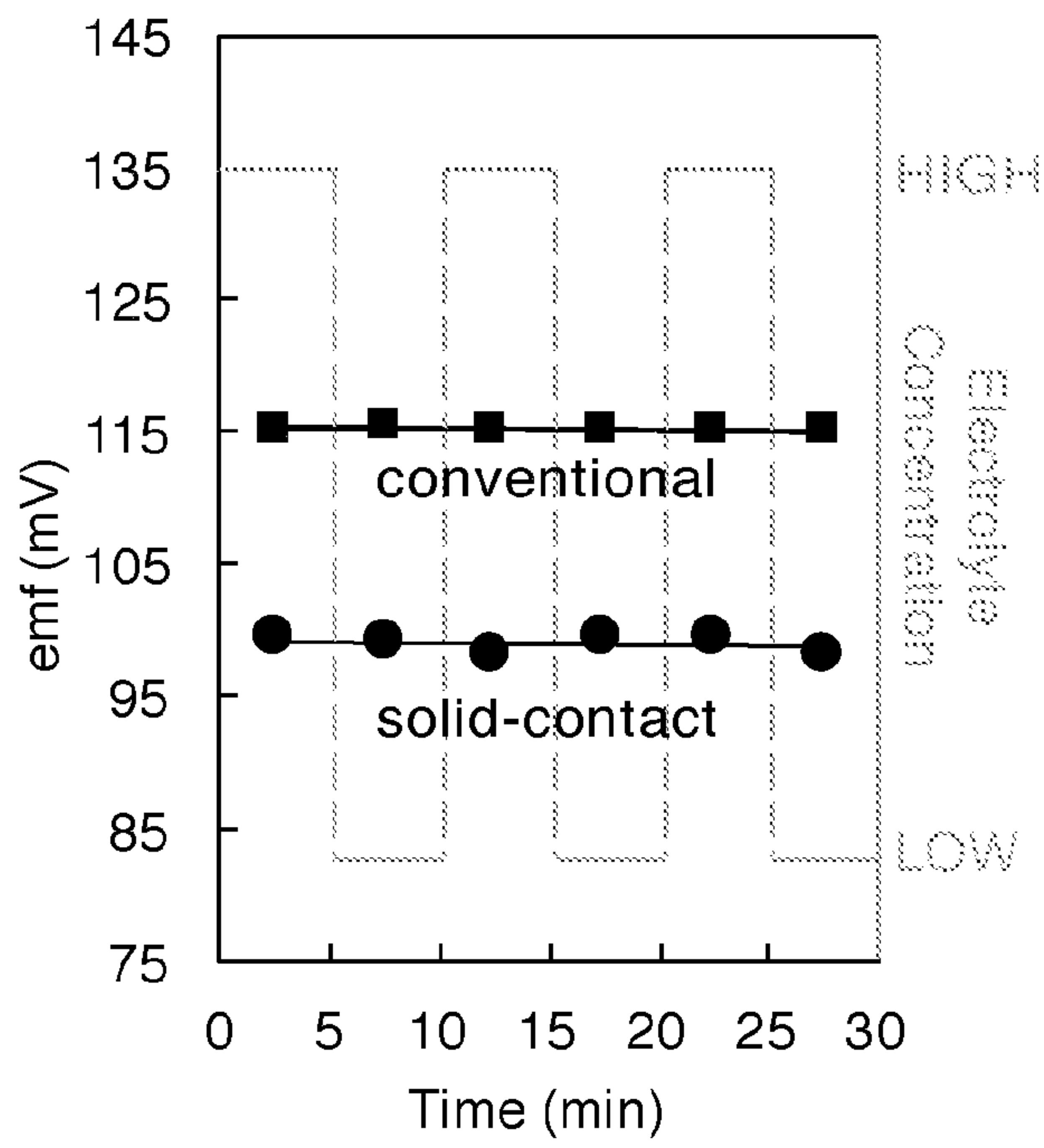


Figure 3

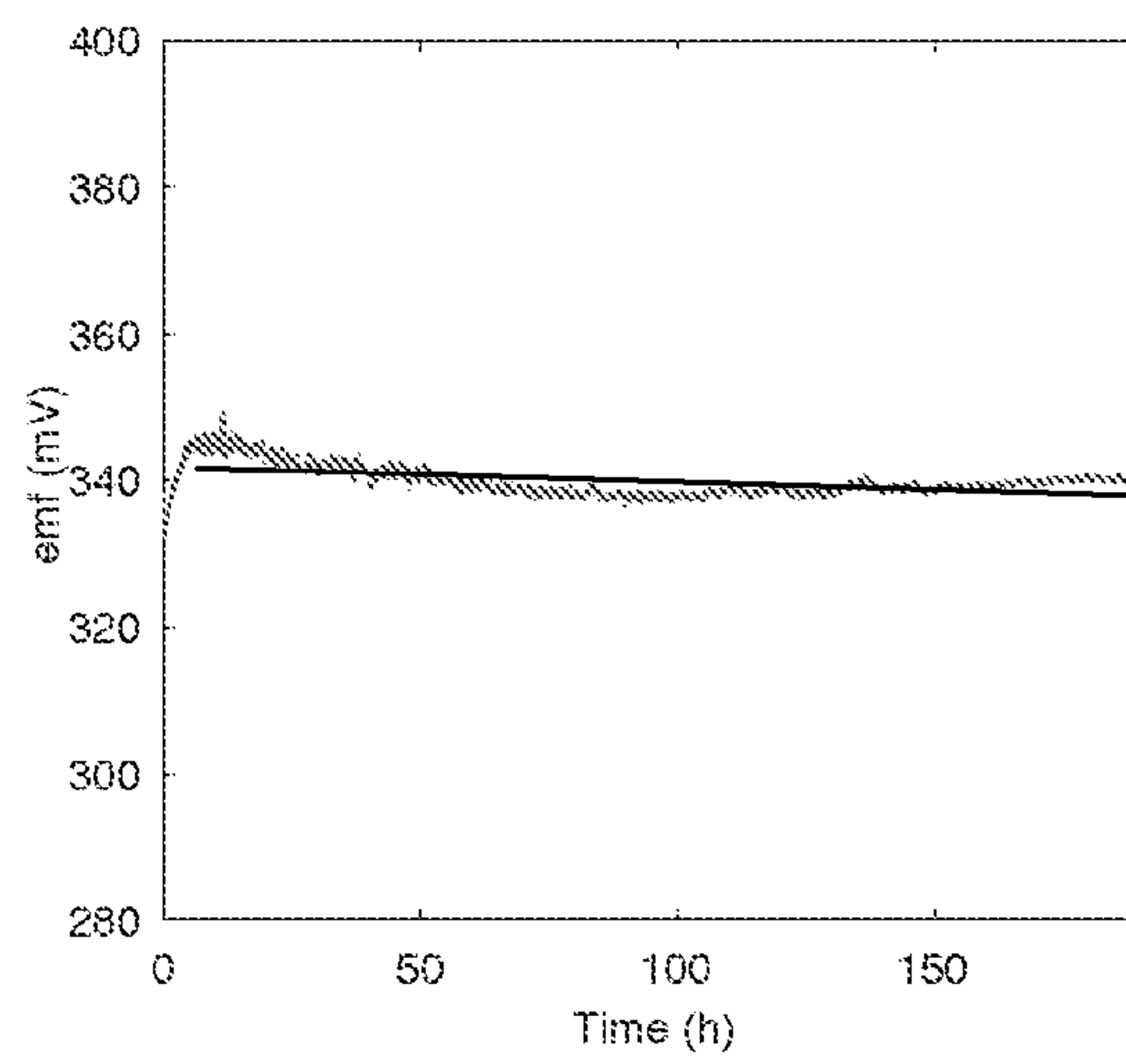


Figure 4

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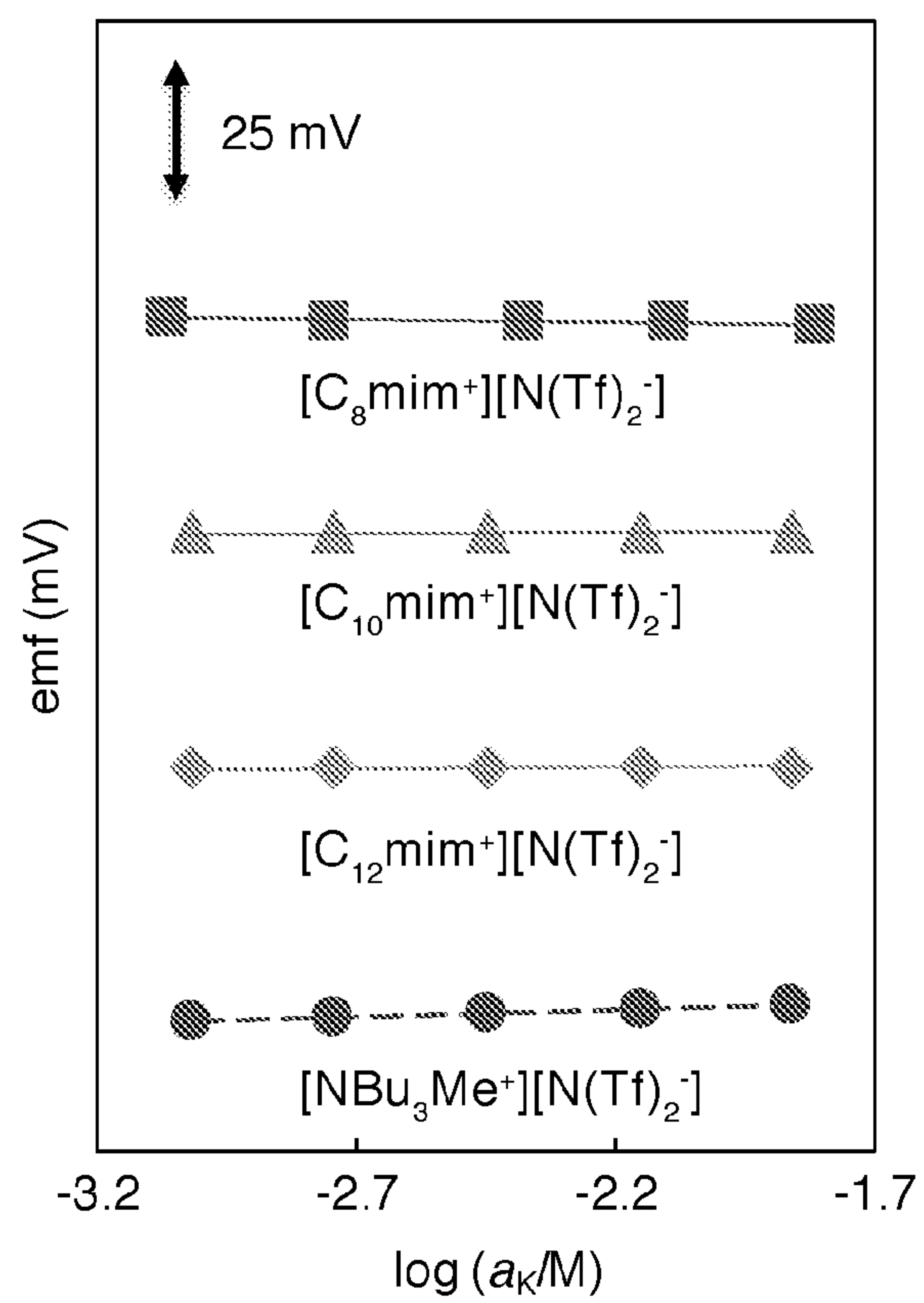


Figure 5

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/59979

A. CLASSIFICATION OF SUBJECT MATTER

IPC - H01M 4/00 (2021.01)

CPC - G01N 27/301, C08L 83/04, C08G 77/04, G01N 27/333, H01M 4/62, H01B 1/02, H01M 10/0565, A61B 5/145, B01L 2300/0645, A61N 1/0404

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2019/0195824 A1 (ENDRESS+HAUSER CONDUCTA GMBH+CO. KG) 27 June 2019 (27.06.2019); the entire document, and more specifically: para [0021], [0051]; figure 1; title; abstract	1-17 and 19-20
A	US 2013/0109790 A1 (FUKUSHI et al.) 2 May 2013 (02.05.2013); the entire document, and more specifically: para [0029], [0054]-[0057], [0062]-[0064], [0088]; title; abstract	1-17 and 19-20
A	US 2006/0065527 A1 (SAMPRONI et al.) 30 June 2006 (30.06.2006); the entire document, and more specifically: para [0001], [0006]-[0009], [0019]-[0021], [0025]; title; abstract	1-17 and 19-20
A	KWON et al., "An all-solid-state reference electrode based on the layer-by-layer polymer coating"; Analyst, Volume 132, Issue 9 (July 2007), pg 906-921 (the entire document, and more specifically: pg 908, col 1, para 2-3; pg 909, col 2, para 1; abstract; table 1)	1-17 and 19-20
A	AHMAD et al., "Role of fumed silica on ion conduction and rheology in nanocomposite polymeric electrolytes"; Polymer, Volume 47, Issue 10 (May 2006), pg 3583-3590 (the entire document, and more specifically: pg 3854, col 1, para 2; pg 3854, col 2, para 3-4; pg 3855, col 1, para 3-5; pg 3856, col 1, para 2; abstract; figure 1; table 1)	1-17 and 19-20
A, P	CHEN et al., "Reference Electrodes Based on Ionic Liquid-Doped Reference Membranes with Biocompatible Silicone Matrixes"; ACS Sensors, Volume 5, Issue 6 (May 2020), pg 1717-1725 (the entire document)	1-17 and 19-20

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"D" document cited by the applicant in the international application	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"E" earlier application or patent but published on or after the international filing date	"&" document member of the same patent family
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 5 January 2021	Date of mailing of the international search report 08 FEB 2021
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Lee Young Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 20/59979

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. Claims Nos.: 18 and 21
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- No protest accompanied the payment of additional search fees.