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(54) Title: SULFONATED TETRAFLUOROETHYLENE BASED IONOMER COATED CARBON FIBER

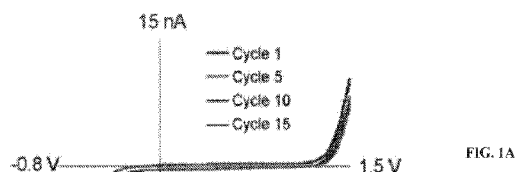


FIG. 1A

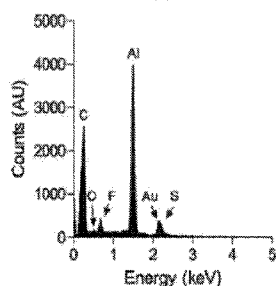


FIG. 1B

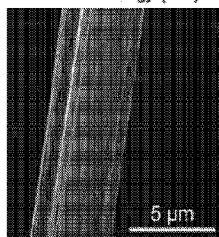


FIG. 1C

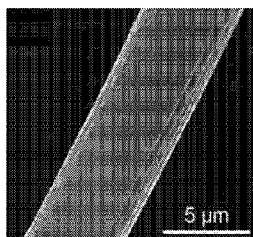


FIG. 1D

(57) Abstract: The present invention provides a carbon fiber that is coated with a composite material. The composite material coating comprises a sulfonated tetrafluoroethylene based ionomer. The present invention also provides a method for producing and using the same. Some aspects of the invention provide a carbon fiber comprising a substantially uniformly coated composite material. In some embodiments, the term "substantially uniformly coated" refers to having a variation of thickness of the coating of about 25% or less, typically about 20% or less, often about 15% or less, more often about 10% or less, and most often about 5% or less throughout at least about 90%, typically at least about 95% and most often at least 98% of the length of the coated carbon fiber.



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SULFONATED TETRAFLUOROETHYLENE BASED IONOMER COATED CARBON FIBER

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of U.S. Provisional Application No. 62/085,438, filed November 28, 2014, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] The present invention relates to a carbon fiber that is coated with a composite material that comprises a sulfonated tetrafluoroethylene based ionomer, and a method for producing and using the same.

BACKGROUND OF THE INVENTION

[0003] Monitoring real-time dynamics of biogenic amines *in vivo* is essential for understanding the role of chemical communication in cognitive function. These molecules are released at axon terminals in response to salient stimuli and diffuse through the extracellular space where they can act on distal receptors (volume neurotransmission) or are cleared by reuptake or metabolic mechanisms. Dopamine is of particular interest because of its well-established role in reward-based behavior,¹ memory,²⁻⁴ addiction,^{5,6} and movement.^{7,8} Most often, carbon-fiber microelectrodes (CFMEs) are utilized for these electrochemical measurements because of their biocompatibility, small size (5-10 μm in diameter), and favorable electrochemical properties. Furthermore, neurotransmission occurs on the sub-second timescale, thus for a measurement to probe transmitter dynamics, the temporal resolution of the measurement must be on the order of milliseconds. Because of this, CFMEs have been coupled to fast-scan cyclic voltammetry.⁹ The shape and magnitude of the voltammetric peaks can be used to identify the molecules present and their respective concentrations.¹⁰ However, during *in vivo* measurements, the presence of interferents complicates measurements warranting additional modification of the electrode surface to enhance selectivity.¹¹

[0004] For *in vivo* measurements, metabolites such as 3,4-dihydroxyphenylacetic acid (DOPAC) and biosynthetic precursors such as L-3,4-dihydroxyphenylalanine (L-DOPA) will be present near the sensor implantation site, at concentrations that may detrimentally affect accurate measurement of the neurotransmitter of interest.¹² Much work has been directed towards maximizing the selectivity of dopamine over ascorbic acid (AA) and DOPAC.¹³⁻¹⁵

These two molecules share a similar oxidation potential with dopamine, and can be present in concentrations 100-fold in excess of dopamine.¹⁶

[0005] To address this, a variety of electrode coatings have been developed to hinder free diffusion of interferents to the electrode. Among these is NAFION®, a copolymer of polytetrafluoroethylene with perfluorovinyl ether sulfonic acid side chains.¹⁵ The sulfonic acid moiety is stabilized by the electron-withdrawing character of the attached chain, and as such the pK_a of the moiety is estimated at -6, leaving the functional group deprotonated at all physiological pH levels.¹⁷ Without being bound by any theory, it is believed that a negative charge immobilized at the surface of the electrode can restrict the diffusion of anions to the electrode. NAFION® also forms cation-conducting sulfonate networks, which allow the transport of positively charged species to the electrode.¹⁸

[0006] NAFION® is commonly dip-coated or electro-deposited onto electrodes prior to *in vivo* measurement in an attempt to minimize current measured from interferents.^{13-15,19} It has also been successfully used to increase selectivity of adenosine measurements,²⁰ and to reduce the shift in reference electrode potential during chronic implantation.²¹ Ascorbic acid and DOPAC are both negatively charged at physiological pH, and dopamine is positively charged, resulting in a decrease of interferent signal and an increase of analyte signal. Because NAFION® is a fluoropolymer like PTFE (polytetrafluoroethylene), it has a tendency to not adhere well to carbon-fiber surfaces and form non-uniform layers.²⁰ Poor adhesion limits the usefulness of NAFION® coatings. Additionally, a reproducible, robust, and facile means for deposition has not yet been achieved.

[0007] Therefore, there is a need for a substantially uniformly NAFION® coated carbon fiber, and a reliable method for producing the same.

SUMMARY OF THE INVENTION

[0008] Some aspects of the invention provide a carbon fiber comprising a substantially uniformly coated composite material. In some embodiments, the term “substantially uniformly coated” refers to having a variation of thickness of the coating of about 25% or less, typically about 20% or less, often about 15% or less, more often about 10% or less, and most often about 5% or less throughout at least about 90%, typically at least about 95% and most often at least 98% of the length of the coated carbon fiber. In some embodiments, the coated composite material (sometimes simply referred to herein as “composite material”) comprises a sulfonated tetrafluoroethylene based ionomer. In one particular embodiment, the sulfonated tetrafluoroethylene based ionomer comprises ethanesulfonyl fluoride, 2-[1-[difluoro-[(trifluoroethenyl)oxy]methyl]-1,2,2,2-

tetrafluoroethoxy]-1,1,2,2,-tetrafluoro-, with tetrafluoroethylene, tetrafluoroethylene-perfluoro-3,6-dioxo-4-methyl-7-octenesulfonic acid copolymer, or a mixture thereof. In another embodiment, the composite material comprises NAFION® and a thiophene polymer. In some instances of this embodiment, the thiophene polymer comprises poly(3,4-ethylenedioxythioph-ene (PEDOT).

[0009] While the carbon fibers of the invention can be used in a wide variety of applications that use uncoated or coated carbon fibers, in one particular embodiment, the carbon fiber of the invention is configured for use as an electrode. In one specific instance, the carbon fiber is configured for use as a microelectrode.

[0010] Other aspects of the invention provide a method for producing a carbon fiber that is coated with a composite material. The composite material includes a sulfonated tetrafluoro-ethylene based ionomer and a thiophene polymer. The method generally includes placing a carbon fiber in a solution comprising a thiophene monomer and a sulfonated tetrafluoroethylene based ionomer in an organic solvent; and applying electricity to said solution under conditions sufficient to produce a carbon fiber comprising a coating of a sulfonated tetrafluoroethylene based ionomer.

[0011] In some embodiments, the coated composite material (e.g., a composite material coated carbon fiber) further comprises a thiophene polymer that is derived from polymerization of thiophene monomer that is present in the solution.

[0012] Another aspect of the invention provides a method for increasing adhesion between carbon fibers and carbon fiber reinforced polymer. Such a method includes using the composite material coating of the invention to increase adhesion between carbon fibers and carbon fiber reinforced polymer. Methods of the invention as well as the carbon fibers of the invention can be used to overcome various shortcomings associated with adhesion difficulties encountered in producing carbon fibers with polymer coatings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 illustrates electropolymerization of EDOT with NAFION® counterions on carbon-fibers. Panel A shows voltammetric trace used for PEDOT:NAFION® coating on a carbon-fiber. Panel B shows energy-dispersive X-ray spectroscopy of a low-density PEDOT: NAFION® coating on a T-40 carbon-fiber indicating the presence of fluorine and sulfur. Panel C is electron micrograph of uncoated carbon-fiber with characteristic ~100 nm striations. Panel D is electron micrograph of low-density PEDOT:NAFION® coated T-40 carbon-fiber.

[0014] Figure 2 shows electrochemical performance of PEDOT:NAFION® coated carbon-fibers. Panel A shows a comparison of low-density PEDOT:NAFION® coated electrodes with uncoated carbon fiber. Panel B shows a comparison of high-density PEDOT:NAFION® coated electrodes. *Left* - background currents recorded at a 75 μm long T-40 carbon-fiber microelectrodes. *Center* - background subtracted voltammograms of 1.0 μM dopamine in a CSF. *Right* - current vs. time traces of a background-subtracted 1.0 μM bolus of dopamine.

[0015] Figure 3 is cyclic voltammetry of dopamine (Panel A), DOPAC (Panel B), and ascorbic acid (Panel C), at PEDOT:NAFION® coated electrodes. Solid line – low-density PEDOT:NAFION® coated electrode, dashed line – uncoated electrode. Cyclic voltammograms were collected with a scan rate of 10 mV/s for dopamine (1 mM), DOPAC (1 mM), and AA (1 mM). Representative background-subtracted fast-scan cyclic voltammograms of dopamine (1.0 μM , Panel D), DOPAC (20 μM , Panel E), and ascorbic acid (200 μM , Panel F) show that the sensitivity for DOPAC and AA has decreased. Electrode sensitivity has increased for dopamine for PEDOT:NAFION® coated vs uncoated electrodes.

[0016] Figure 4. shows chemical selectivity of PEDOT:NAFION® coated, NAFION® dip-coated, and uncoated carbon-fibers for dopamine over ascorbic acid and DOPAC. Selectivity is defined as an equimolar current ratio at the peak oxidation potential of dopamine. Error bars are SEM ($n = 3 - 15$).

[0017] Figure 5 shows PEDOT:NAFION® electrodes resist *in vitro* (Panel A) and *in vivo* (Panel B) biofouling. Panel C is a representative uncoated CFME showing a large accumulation of biomaterial on the electrode after 30 minutes of implantation in the nucleus accumbens. Panel D is a low-density PEDOT:NAFION® coated carbon fiber implanted for six hours showing decreased adsorption of biomaterial when compared to uncoated carbon fibers.

[0018] Figure 6 shows *in vivo* measurements of dopamine release using a PEDOT:NAFION® coated carbon-fiber microelectrode. Current vs. time traces are extracted from the color plot at the peak oxidation potential for dopamine (600 mV). Representative cyclic voltammograms are taken from the vertical white dashed line on the color plots. Panel A: dopamine release was electrically evoked by stimulation of the medial forebrain bundle and monitored in the nucleus accumbens of a Sprague-Dawley rat. Panel B: spontaneous transients recorded in the nucleus accumbens of an anesthetized Sprague-Dawley rat.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The invention generally relates to a carbon fiber comprising a substantially uniformly coated composite material and a method for producing and using the same. The coated composite material typically comprises a sulfonated tetrafluoroethylene based ionomer. In some embodiments, the term “substantially uniformly coated” refers to having about 15% or less, typically about 10% or less, and often about 5% or less of thickness variation of the coated composite material at about 5, typically at about 10 and often at about 15 randomly or arbitrarily selected positions. The term “about” refers to $\pm 20\%$, typically $\pm 10\%$, and often $\pm 5\%$ of the numeric value. The term “coated” refers to having the composite material attached to carbon fiber such that at standard conditions (e.g., 20 °C at 1 atmosphere of pressure), at least 95% of the composite material remains attached to (e.g., does not flake-off or peel-off from) the carbon fiber for at least about 7 days, typically for at least about two weeks, often for at least one month, and most often for at least six months.

[0020] The sulfonated tetrafluoroethylene based ionomer is typically positively charged, i.e., a cation. Exemplary sulfonated tetrafluoroethylene based ionomers include, but are not limited to, a copolymer of polytetrafluoroethylene with perfluorovinyl ether sulfonic acid side chains, such as ethanesulfonyl fluoride, 2-[1-[difluoro-[(trifluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-, with tetrafluoroethylene, tetrafluoroethylene-perfluoro-3,6-dioxo-4-methyl-7-octenesulfonic acid copolymer and the like. Typically, such sulfonated tetrafluoroethylene based ionomers are known to one skilled in the art as NAFION®. The composite material of the invention can also include a polymer of a thiophene monomer. In one particular embodiment, the thiophene compound is ethylenedioxythiophene (EDOT).

[0021] In one specific embodiment, the composite material of the invention comprising NAFION® is coated onto carbon-fiber microelectrodes by synthesizing a polymer comprising polyethylenedioxythiophene (PEDOT) and NAFION® on the surface of the carbon fiber. Methods of the invention provide a substantially uniform thin layer of a surface-immobilized composite material. Unless context requires otherwise, the terms “coating” and “surface-immobilized” are used interchangeably herein and refer to having the composite material bound to the surface of the carbon fiber. The composite material may be attached to the carbon fiber by covalent bonding, ionic bonding, hydrophobic interaction, due to Van Der Waal’s force, or any other chemical and/or physical means as long as the coated composite material is stable for at least about 10, typically at least about 20, often at least about 50 and most often at least about 100 intended use. Alternatively, the carbon fiber is

considered to be coated with the composite material as long as the performance of the coated carbon fiber does not diminish more than about 20%, typically no more than about 15%, often no more than about 10%, and most often no more than about 5% after about 10, typically after about 20, often after about 50, and most often after about 100 uses.

[0022] The thickness of the coated composite material can vary depending on a particular application. For example, for *in vivo* dopamine measurement, the thickness of the composite material is at least about 50 nm, typically at least about 100 nm, often at least about 200 nm, and most often at least 300 nm. Alternatively, the thickness of the composite material for *in vivo* dopamine measurement range from about 50 nm to about 500 nm, typically from about 50 nm to about 400 nm, often from about 75 nm to about 300 nm and most often from about 100 nm to about 250 nm. However, it should be appreciated that the coated composite material of the invention is not limited to these specific thicknesses and examples given herein. The thickness of the composite material that is coated onto the carbon fiber can vary in order to affect the desired properties such as, selectivity, specificity, durability, cost of fabrication, etc.

[0023] In some particular embodiments, a thin substantially even or uniform coating of the composite material exhibits a comparatively high chemical selectivity, e.g., relative to a non-coated carbon fiber. With regards to a specific composite material comprising PEDOT and NAFION®, both of these materials have a well-established history in being coated onto biosensors to improve sensor function or biocompatibility,²⁵⁻²⁹ As discussed herein, the present disclosure provides a method for deposition of a PEDOT:NAFION® composite material onto a carbon fiber and characterization of the coated composite material. The present invention also describes a facile process for coating the composite material onto a carbon fiber. Such coated carbon fiber produced accurate measurements of dopamine *in vivo*. Additionally, coating with the composite material of the invention provided a carbon fiber surface that was significantly resistant to biofouling and retained enhanced selectivity and sensitivity for dopamine over interferents following six hours of *in vivo* implantation.

[0024] One specific aspect of the invention provides a carbon fiber comprising a substantially uniformly coated composite material. The coated composite material comprises a sulfonated tetrafluoroethylene based ionomer. In some embodiments, the sulfonated tetrafluoroethylene based ionomer comprises ethanesulfonyl fluoride, 2-[1-[difluoro-[(trifluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-, with tetrafluoroethylene, tetrafluoroethylene-perfluoro-3,6-dioxo-4-methyl-7-octenesulfonic acid copolymer, or a mixture thereof.

[0025] In one particular embodiment, the composite material comprises NAFION® and a thiophene polymer. In some instances, the thiophene polymer comprises poly(3,4-ethylenedioxythiophene (PEDOT). Still in other embodiments, the carbon fiber is configured for use as an electrode. In some cases, the carbon fiber is configured for use as a microelectrode. Yet in another embodiment, the thickness of coated composite material in the microelectrode is about 100 nm.

[0026] In some embodiments, the carbon fiber of the invention comprising a coated composite material has an increased sensitivity and/or selectivity to an amine compound. That is, compared to a similar carbon fiber that is not coated, the sensitivity and/or selectivity of the coated carbon fiber microelectrode is at least about 5%, typically at least about 10%, often at least about 20% more often at least about 50%, and most often at least about 100% more selective and/or sensitive to an amine compound, such as dopamine.

[0027] Another aspect of the invention provides a method for producing a carbon fiber comprising a coated composite material that includes a sulfonated tetrafluoroethylene based ionomer. The method generally includes placing a carbon fiber in a solution comprising a thiophene monomer and a sulfonated tetrafluoroethylene based ionomer in an organic solvent; and applying electricity to said solution under conditions sufficient to produce a carbon fiber comprising a coating of a sulfonated tetrafluoroethylene based ionomer. In some embodiments, the electricity is applied in a triangular potential waveform, e.g., using cyclic voltammetry. In other embodiments, the composite material further comprises a thiophene polymer, where the thiophene polymer is derived from polymerization of a thiophene monomer. In some embodiments, the thiophene monomer comprises ethylenedioxythiophene (EDOT). Still yet in other embodiments, the composite material comprises the sulfonated tetrafluoroethylene based ionomer and a polymer of the thiophene monomer.

[0028] In one particular embodiment, a composite material comprising NAFION® and PEDOT has been coated onto carbon-fiber microelectrodes electrochemically, e.g., via electrolysis of a solution comprising NAFION® and EDOT. Such a composite material provides a mechanically stable, robust, and controllable electrode coating that showed increased selectivity and/or sensitivity of electrochemical measurements *in vivo*. One particular method of producing the composite material coating is to deposit the composite material onto carbon-fiber microelectrodes by applying a triangle waveform from + 1.5 V to – 0.8 V and back in a dilute solution of ethylenedioxythiophene (EDOT) and NAFION® in

an organic solvent such as acetonitrile. It should be appreciated that other organic solvents can also be used.

[0029] Scanning electron microscopy (SEM) was used to demonstrate that the coating is uniform and approximately 100 nm thick. Energy-dispersive x-ray spectroscopy (EDX) demonstrated that both sulfur and fluorine are present in the composite material coating, indicating the incorporation of PEDOT (poly(3,4-ethylenedioxythiophene) and NAFION®. The amount or the thickness of the coated composite material can be modified as desired, e.g., by varying the EDOT and/or NAFION® concentration. In addition to SEM and EDX described above, the composite material coating can also be characterized electrochemically.

[0030] The PEDOT:NAFION® composite material coated carbon fibers made using 200 μ M EDOT exhibited an average 10-90 response time of 0.46 ± 0.09 seconds vs. 0.45 ± 0.11 seconds for an uncoated fiber in response to a 1.0 μ M bolus of dopamine. The electrodes coated using a higher EDOT concentration (400 μ M) are slower with an average 10-90 response time of 0.84 ± 0.19 seconds, but had an increased sensitivity to dopamine, e.g., 46 ± 13 nA/ μ M compared to 26 ± 6 nA/ μ M for the electrodes coated in 200 μ M EDOT and 13 ± 2 nA/ μ M for an uncoated fiber. In addition to improvements in sensitivity and selectivity, the coating showed dramatically reduced acute biofouling. When PEDOT:NAFION® coated electrodes were lowered into the nucleus accumbens of a rat, both spontaneous and stimulated release and reuptake of dopamine can be measured.

[0031] Additional objects, advantages, and novel features of this invention will become apparent to those skilled in the art upon examination of the following examples thereof, which are not intended to be limiting. In the Examples, procedures that are constructively reduced to practice are described in the present tense, and procedures that have been carried out in the laboratory are set forth in the past tense.

EXAMPLES

[0032] *Electrode fabrication:* Each carbon fiber microelectrode (i.e., CFME) was fabricated by isolating and aspirating a single T-40 carbon fiber (Cytec Thornel, Woodland Park, NJ) into a standard glass capillary (1.2 mm o.d. x 0.68 mm i.d., 4" long, A-M Systems, Sequim, WA, USA). Each filled capillary was then heated and pulled using a type PE-2, RR50915300 pipet puller (Narishige, Tokyo, Japan). The carbon-fiber electrode was cut to ~ 75 μ m in length from the glass seal using a surgical blade. Electrical contact was made by inserting wire-wrap wire coated in alcohol-based graphite conductive adhesive (1.2 kOhm/in², Alfa Aesar, Ward Hill, MA) through the open end of the capillary tube. An epoxy seal using Loctite 1C Hysol epoxy-patch adhesive (Henkel Corporation, Madison Heights,

WI, USA) was made around the periphery of the electrical wire protruding from the glass capillary and cured overnight at room temperature. For method validation, some electrodes were also successfully fabricated with AS-4 and IM-7 type carbon fibers (Hexcel Corporation, Stamford, CT, USA) to ensure that this chemistry was not specific to T-40 carbon fibers. T-40 carbon fibers were used for all electrodes characterized electrochemically.

[0033] *Chemicals:* Electrodes were pre-tested prior to coating deposition in an artificial cerebrospinal fluid solution (aCSF) (15 mM Tris HCl, 126 mM NaCl, 2.5 mM KCl, 20 mM Na₂CO₃, 1.2 mM NaH₂PO₄, 2.0 mM Na₂SO₄, 2.4 mM CaCl₂, 1.2 mM MgCl₂, pH = 7.40). Prior to CaCl₂ and MgCl₂ addition, the pH of the aCSF was adjusted to 7.40 using 0.1 N NaOH or a 0.1 N HCl solution. Electrodes were submerged in buffer and a triangle waveform from - 0.4 V to + 1.3 V was applied at 400 V/s for 1 minute; electrodes without a stable background were discarded. PEDOT:NAFION® deposition solutions consisted of 100-200 µL of a stock solution of 0.04 M EDOT (Sigma Aldrich, St. Louis, MO, USA) in acetonitrile (prepared by the addition of 43 µL EDOT to 10 mL acetonitrile) and 200 µL of LQ-1105 NAFION® (Ion Power Inc., DE, USA) in 20 mL acetonitrile (HPLC grade, EMD Chemicals Inc., Darmstadt, Germany). The final deposition solutions prepared from the stock solution contained either 200 µM EDOT (low-density PEDOT:NAFION® coating) or 400 µM EDOT (high-density PEDOT:NAFION® coating). Prior to electrodeposition, deposition solutions were mixed for 1 minute and used within 12 hours. Dopamine, ascorbic acid, DOPAC, bovine serum albumin, and all other chemicals, unless otherwise specified, were purchased from Sigma Aldrich. Neurotransmitter measurements were performed in aCSF buffer solution.

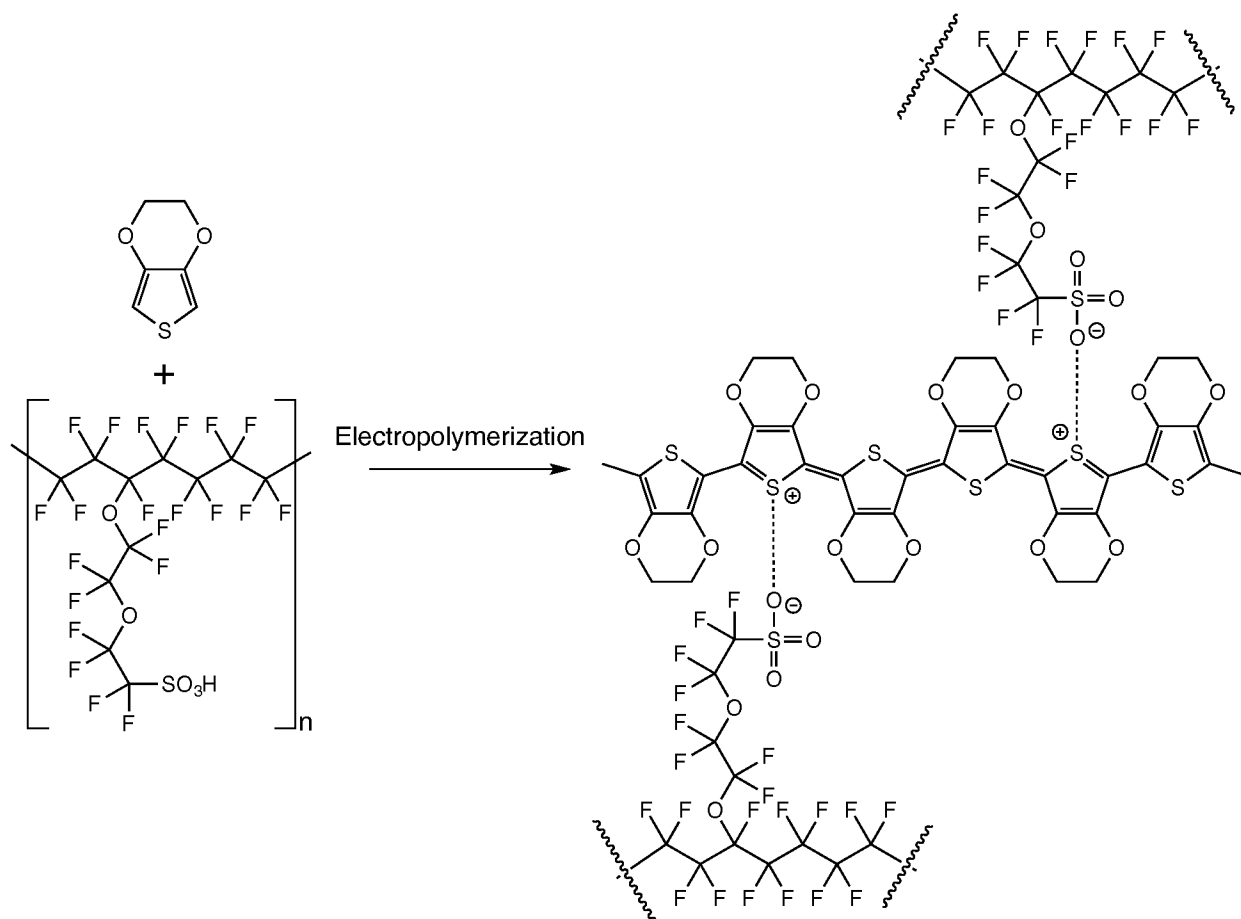
[0034] *Electrochemistry:* The voltage for electrodeposition was controlled using a Gamry Instruments Reference 600 potentiostat (Warminster, PA, USA) in a three-electrode configuration. A tightly coiled silver wire was used as the counter electrode, and a straight silver wire was used as the reference electrode. Both the reference and counter electrodes were polished using sandpaper and rinsed using 18.2 MΩ doubly-deionized (MilliQ) water. Deposition was performed by applying a triangle waveform from +1.5 V to -0.8 V at 100 mV/s for 15 cycles, and using an open-circuit potential between waveform application. Electrochemical characterization of coated and uncoated electrodes was performed via fast-scan cyclic voltammetry using the WCCV 3.0 software package, (Knowmad Technologies, LLC, Tucson, Arizona). A programmatically controlled a flow cell 6-port valve switch (VICI Valco, Houston, TX, USA) and Dagan ChemClamp potentiostat (Minneapolis, MN,

USA) for background-subtracted electrochemical measurements of neurotransmitters and interferents.

[0035] *Biological Experiments:* Adult, male Sprague-Dawley rats (350 – 450 g; Harlan Laboratories, Harlan, Kentucky, USA) were used. All procedures were performed in accordance with the policies of the National Institutes of Health guidelines for laboratory animals.

[0036] **Results and Discussion:** The polymerization of monothiophenes such as ethylenedioxythiophene (EDOT) is possible through a variety of oxidant-initiated or electrochemical processes.³¹⁻³⁴ Commonly, iron (iii) tosylate (the monomeric homologue of polystyrenesulfonate) or iron (iii) chloride are used as oxidants for *in situ* polymerization.³⁵ Solutions of EDOT and oxidant in solvent are deposited, dried, and rinsed to form a conductive polymer film. Alternatively, EDOT can be oxidatively electro-polymerized to PEDOT in the presence of a counter ion. NAFION® contains sulfonate groups much like iron tosylate or polystyrenesulfonate making it a suitable counter ion. Additionally, because oxidized PEDOT is positively charged, NAFION® can be incorporated into the coating as a counter ion. Given this similarity, thick PEDOT:NAFION® composite films have been previously synthesized on platinum wires via a galvanostatic deposition in a 5% aqueous dispersion of NAFION® with small a volume of EDOT added, though to date, no applications of this composite material have been described.³⁶

[0037] Described herein is the electro-synthesis of a surface-immobilized PEDOT:NAFION® composite polymer. The present inventors have developed appropriate solution concentrations to examine two different coating regimes, called low-density PEDOT:NAFION® and high-density PEDOT:NAFION®. Additionally, the present inventors have implemented an information-rich coating deposition method, cyclic voltammetry, which has been shown to increase the nucleation density of electropolymerized conducting polymers when compared to an amperometric deposition.³⁷ This appears to result in an increased NAFION® density (and thus an increased repulsion of anionic species) at the electrode. Without being bound by any theory, it is believed that the final structure of the PEDOT has a positive charge which is coordinated by a NAFION® sulfonate as schematically illustrated in Scheme 1.



Scheme 1

[0038] A positive charge every three monomer units has been experimentally verified for PEDOT:Tosylate and given the similarity of the oxidant, it is expected that the NAFION® sulfonate coordinates similarly.³⁸ Furthermore, measurements made at other electrochemically polymerized PEDOTs with sulfonate dopants indicate that an excess of sulfonates is present in the coatings. Extending this conclusion, the PEDOT:NAFION® coating is likely not charge neutral (*i.e.*, one sulfonate to one PEDOT positive charge) but instead contains an excess of sulfonate groups relative to the positive charges on PEDOT.³⁸ Given that a PEDOT-associated sulfonate on a NAFION® chain is only one of many, this may increase the negative character of the coating, generating selectivity towards cations

[0039] An ordinary deposition trace is shown, with cycles 1, 5, 10, and 15 highlighted in Figure 1. Three prominent characteristics of this deposition are apparent. First, an oxidation current near the anodic limit (+1.5 V) is attributed to the oxidation of EDOT that polymerized to form PEDOT. This oxidation current on the deposition voltammogram can be considered the film-forming current, serving as an indicator of coating success. Doubling the EDOT concentration during a deposition resulted in a larger oxidative deposition current. Second, a reduction wave starting near -0.6 V is apparent. This wave has been attributed to

the reduction of protons into molecular hydrogen. The cycling of a carbon-fiber microelectrode using a deposition waveform in a solution of acetonitrile and sulfuric acid results in a similar wave shape and potential. Third, a small irreversible peak-shaped wave is apparent at 50 mV, and appears in most deposition traces. We attribute this peak to an irreversible oxidation of the PEDOT coating, as observed elsewhere in PEDOT electro-synthesis literature.^{39,40} Voltammetric deposition of EDOT without the presence of a counter ion in solution does not form a coating or generate an oxidative current.

[0040] Scanning electron microscopy was used to compare the surface morphology of uncoated and PEDOT:NAFION® coated carbon-fiber microelectrodes (Figure 1). The unmodified carbon fiber prominently exhibited a striated surface, with individual striations measuring between 50 and 200 nm wide. After deposition of a low-density PEDOT:NAFION® coating on the electrode, a ~100 nm coating on the electrode obfuscated the striations, and imparted a smooth surface morphology. Energy-dispersive X-ray spectroscopy was utilized to measure the presence of sulfur and fluorine in the coatings. The fluorine K α line was used to confirm the presence of NAFION® in the PEDOT:NAFION® coatings (Figure 1). As can be seen, the fluorine peak is present for polymer-coated electrodes, and is absent for uncoated electrodes. Interestingly, given a constant NAFION® concentration and an increasing EDOT concentration, the fluorine peak grows with higher EDOT concentrations, indicating that the PEDOT incorporates more counterion NAFION® into the coating if there is more PEDOT polymerized during the voltammetric deposition process.

[0041] To explore the chemical effect of EDOT concentration on coating performance, two concentrations of EDOT (and thus two coating types) were chosen for exploration. The first coating type (Figure 2, Panel A) was prepared with the intent of maximizing selectivity while minimizing changes in temporal response or background current using the previously described deposition waveform in a 20 mL solution of acetonitrile containing 100 μ L EDOT stock (0.04 M EDOT in acetonitrile) and 200 μ L NAFION®. This scheme is referred to as “low-density PEDOT:NAFION®”. Flow-injection analysis background-subtracted fast-scan cyclic voltammetry was used to characterize the effect of low-density PEDOT:NAFION® coatings on the temporal response and background current of the electrode, and this data is shown in Table 1 and Figure 2, respectively.

Table 1. Figures-of-merit for uncoated and PEDOT:NAFION® electrodes acquired via flow-injection analysis with background-subtracted fast-scan cyclic voltammetry (n = 6 electrodes, $\pm$ SEM).

Electrode Type	Rise time	DA Sensitivity	RMS Noise (pA)	DA LOD
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	(s)	(nA/ μ M)		(nM)
Uncoated	0.45 ± 0.11	13 ± 2	80 ± 30	20 ± 7
PEDOT:NAFION® 200 μ M EDOT	0.46 ± 0.09	26 ± 6	30 ± 10	4 ± 1
PEDOT:NAFION® 400 μ M EDOT	0.84 ± 0.19	46 ± 13	100 ± 40	6 ± 1

An increase in sensitivity of from 13 ± 2 nA/ μ M for uncoated carbon fibers to 26 ± 6 nA/ μ M for coated carbon fibers was observed, and this difference was statistically significant (Student's t-test, $n = 6$ electrodes, $P < 0.01$). The 10-90 rise time of a 1 μ M bolus of dopamine detected at the low-density PEDOT:NAFION® coated electrode was 0.45 ± 0.11 seconds, compared to 0.46 ± 0.09 seconds from an uncoated electrode. This difference was not statistically significant (Student's t-test, $n = 6$ electrodes, $P = 0.95$). The background current of an uncoated electrode was recorded, followed by the deposition of a coating on that electrode, and then the background was recorded post-deposition. The background shape and current were essentially unchanged. The second coating type (Figure 2, Panel B), referred to as high-density PEDOT:NAFION®, was prepared with the intent of maximizing selectivity and sensitivity while maintaining a background current under the maximum current threshold of typical FSCV headstages. The high-density PEDOT:NAFION® coating resulted in a 4-fold increase in sensitivity with respect to the uncoated carbon-fiber electrode for dopamine, at 46 ± 13 nA/ μ M. This difference was statistically significant (Student's t-test, $n = 6$ electrodes, $P < 0.05$). The background current increases nearly 3-fold, though the background shape was similar. The wave shape of a background-subtracted dopamine voltammogram was markedly different as the oxidative current was ~ 4 times larger while the reduction current increased only by a factor of 1.5 compared to a control electrode. It is possible that differences in proton-transfer equilibrium between the adsorbed dopamine and adsorbed dopamine-orthoquinone may have given rise to this effect. The 10-90 rise time of a 1.0 μ M bolus of dopamine detected at the higher EDOT coated electrode was 0.84 ± 0.19 seconds, compared to 0.46 ± 0.09 seconds from an uncoated electrode. Clearly, a sacrifice in temporal resolution was made for a substantial increase in sensitivity. This temporal resolution may not be needed for all types of measurements, for example, in equilibrium surface coverage measurements of dopamine via fast-scan controlled-adsorption voltammetry, but may be advantageous for low signal recordings, such as *in vivo* measurements of spontaneous phasic dopamine release, or perhaps for lower concentration transmitters.

[0042] NAFION® is a negatively charged polymer which has been used extensively for increasing the selectivity for cations *in vivo*.^{19,20,41–43} Following deposition of NAFION® onto the electrode surface it is expected that mass transfer for a cation (such as dopamine) should be faster than that of an anion (such as DOPAC or ascorbic acid). To validate that the PEDOT:NAFION® coating is decreasing the rate of mass-transfer of DOPAC and ascorbic acid, following electrodeposition of the PEDOT:NAFION® coating onto the carbon-fiber microelectrode, slow scan cyclic voltammetry (-0.4 V to 1.0 V at 20 mV/s) was performed on solutions of 1.0 mM dopamine, DOPAC, and AA in 20 mM pH 7.4 phosphate buffered saline (Figure 3). As can be seen in Figure 3A, the steady-state current was not significantly changed for the coated electrodes, indicating that the coating had no effect on the rate of mass transfer for dopamine. However, a considerable decrease was apparent in both Figure 3B and 3C which correspond to two DOPAC and ascorbic acid (anions at physiological pH). The electron micrographs of the high-density PEDOT:NAFION® coated electrodes do not indicate an increased geometric electrode area.

[0043] Selectivity of PEDOT:NAFION® coated electrodes for dopamine over two negatively charged interferents (DOPAC and ascorbic acid) was quantified by flow-injection analysis of background-subtracted fast-scan cyclic voltammetry (Figure 4). Equimolar current ratios of dopamine and DOPAC or dopamine and ascorbic acid were used to calculate the selectivity of the sensor. The current used in this ratio was measured at peak potential of the oxidation wave for dopamine. Uncoated electrodes offer a selectivity of 54 ± 6 for dopamine/AA and 21 ± 4 for dopamine/DOPAC. Electrodes prepared using a traditional dip-coating method¹⁹ exhibited statistically insignificant increases to 97 ± 20 for dopamine/AA and 23 ± 7 for dopamine/DOPAC (Student's t-test, $n = 3$ electrodes, $P > 0.1$ for both). The low-density PEDOT:NAFION® coating offered a statistically significant increase in selectivity for DA/AA at an average of 534 ± 57 (Student's t-test, $n = 15$ electrodes, $P < 0.05$), and at high-density PEDOT:NAFION® coated electrodes, the selectivity increased to 1540 ± 145 which was also statistically significant compared to uncoated carbon-fiber electrodes (Student's t-test, $n = 3$ electrodes, $P < 0.05$). The selectivity for DA/DOPAC was increased from 21 ± 4 (uncoated) to 45 ± 6 (low-density PEDOT:NAFION® coating) and 52 ± 4 (high-density PEDOT:NAFION® coating). This difference was statistically significant when comparing uncoated to any of the PEDOT:NAFION® coated electrodes (Student's t-test, $n = 3 - 15$ electrodes, $P < 0.05$).

[0044] Electrode noise was characterized on 15 low-density PEDOT:NAFION® and 3 high-density PEDOT:NAFION® coated electrodes by measuring the RMS (root mean

square) current noise of the electrode between 0.575 and 0.625 V vs. Ag/AgCl while scanning at 400 V/s in pH 7.4 Tris buffer. Resultant data was filtered as previously described⁴⁴ These data were hardware low pass filtered at 10 kHz and software 4-pole Butterworth zero-phase low pass filtered at 2 kHz. The RMS noise was for uncoated carbon fibers was 80 ± 30 pA. Low-density PEDOT:NAFION® coated electrodes had a statistically significant decrease in noise to 30 ± 10 pA (Student's t-test, $n = 15$ electrodes, $P < 0.01$), while high-density PEDOT:NAFION® coated electrodes showed a statistically significant increase to 100 ± 40 pA (Student's t-test, $n = 3$ electrodes, $P < 0.01$).

[0045] Background-subtracted fast-scan voltammetry was also used to measure limit-of-detection for dopamine at uncoated, low-density PEDOT:NAFION® coated, and high-density PEDOT:NAFION® coated electrodes. The limit-of-detection was calculated by measuring the RMS current noise across the oxidation potential for dopamine over a 50 mV window (*vide supra*, Table 1). For uncoated carbon-fiber microelectrodes, this was 20 ± 7 nM. Low-density PEDOT:NAFION® coated electrodes had a dopamine limit of detection of 4 ± 1 nM, while high-density PEDOT:NAFION® coated electrodes had a dopamine limit of detection of 6 ± 1 nM. Both of these limits of detection for PEDOT:NAFION® coated carbon-fiber electrodes were statistically different compared to the uncoated carbon-fiber electrode (Student's t-test, $n = 6$ electrodes, $P < 0.05$).

[0046] The PEDOT:NAFION® coated sensors were characterized *in vitro* and *in vivo* for their ability to resist biofouling. In the context of these experiments, biofouling is defined as the sensor's decrease in sensitivity to dopamine as measured by flow cell background-subtracted FSCV after being placed in a challenging chemical environment. To assess this resistance to biofouling, pre-calibration sensitivity was compared with post-calibration sensitivity. While others have shown that the sensitivity of tyramine-fouled carbon fiber microelectrodes was renewed with application of the + 1.3 V waveform for 15 minutes,⁴⁵ post-calibration was infrequently performed by modern practitioners of *in vivo* voltammetry so biofouling can be difficult to assess. Some studies have indicated that the majority of biofouling occurs in the first 30 minutes of implantation, stabilizing at a decreased sensitivity thereafter.^{14,46–49}

[0047] Fouling of the electrode in biological tissue was simulated by implanting the electrode into 40 g/L solution of bovine serum albumin (BSA) in pH 7.4 Tris aCSF. This solution has been used elsewhere to mimic the fouling capacity of the brain environment.^{49,50} Uncoated and three low-density PEDOT:NAFION® coated CFMEs were submerged in this solution for 2 hours. Three uncoated CFMEs were also submerged in pH 7.4 Tris aCSF and

cycled using the same waveform for two hours as a control for sensor degradation via application of the waveform.

[0048] For the *in vitro* fouling procedure in BSA, uncoated CFMEs lost 40 ± 5 % of their sensitivity to a 1 μ M bolus of dopamine (Figure 5, panel A). Low-density PEDOT:NAFION® coated CFMEs lost only 5 ± 6 % of their sensitivity, and electrodes cycled in pH 7.4 Tris aCSF lose only 1 ± 3 % of their sensitivity. This sensitivity loss of approximately 40% for uncoated fibers has also been reported elsewhere.⁴⁹ The difference between coated and uncoated fibers is statistically different (Student's t-test, $P < 0.01$, $n = 3$), while the difference between low-density PEDOT:NAFION® coated CFMEs and control electrodes in pH 7.4 Tris aCSF is not statistically significant (Student's t-test, $P > 0.3$, $n = 3$). For the *in vivo* fouling procedure, electrodes were implanted in the cortex (uncoated CFME) of a male Sprague-Dawley rat for 30 minutes (uncoated CFMEs) or 2 hours in the nucleus accumbens (coated CFMEs). Uncoated carbon fibers lost an average of 57 ± 38 % of their sensitivity over the course of 30 minutes of implantation (Figure 5, panel B). Low-density PEDOT:NAFION® coated CFMEs lost only 9 ± 5 % of the pre-calibration sensitivity, despite being implanted for four times longer than the uncoated fibers. The origin of this large variability in uncoated CFMEs could be differences in surgical preparation. Recently, two-photon mapping measurements made during implantation of microelectrodes showed that whether the microelectrode ruptures a blood vessel as it is lowered into the cortex can dramatically impact the performance of the microelectrode.⁵¹ Because few laboratories are equipped to guide the microelectrode into the brain with advanced blood vessel imaging techniques, there is value in mitigating fouling originating from ruptured intracranial blood vessels or other sources not described in literature.

[0049] Scanning electron microscopy with energy-dispersive X-ray spectroscopy was also performed to examine the surface morphology and fluorine content of coated and uncoated electrodes post-implantation. A representative uncoated CFME showed a large accumulation of biomaterial, perhaps a blood clot, on the electrode after just 30 minutes of implantation in the cortex (Figure 5, panel C). In contrast, a representative electron micrograph of a low-density PEDOT:NAFION® coated CFME implanted for six hours showed little adsorption of biomaterial on the electrode. The coated electrode was also noticeably smoother in morphology. Because the surface of the electrode is very gradually etched off with application of the +1.3 V.⁴⁵ Despite this etching, the coated electrode was also determined to have retained fluorine (and thus NAFION®) on the electrode surface following implantation for six hours.

[0050] To validate the sensor's response to a typical voltammetric stimulated release experiment, a stainless steel stimulating electrode was implanted in the medial forebrain bundle and a low-density PEDOT:NAFION® coated carbon-fiber microelectrode was implanted in the nucleus accumbens of an anesthetized Sprague-Dawley rat. Prior to implantation, the coated electrode was cycled from -0.4 V to +1.3 V at 400 V/s in pH 7.4 a CSF buffer for 10 minutes. Stimulated release of dopamine was measured with a concentration of approximately 300 nM (Figure 6A). A color plot recorded during stimulation showed typical stimulated release and re-uptake (Figure 6A). Stimulation is marked by a black bar below the color plot. Additionally, a current vs. time trace of the current at the oxidation wave for dopamine, and an extracted voltammogram are shown (Figure 6A, inset). To further validate the use of this sensor *in vivo*, dopamine release was monitored in the nucleus accumbens of an anesthetized rat (Figure 6B). This is believed to be the first recording of anesthetized dopamine transient release events without administration of cocaine. Several dopamine transients ranging from 5 - 200 nM were present. A representative cyclic voltammogram was taken at the white vertical dashed line and displayed in the inset. It is believed that this measurement was only made possible by the increased sensitivity of the PEDOT:NAFION® coated electrodes.

[0051] Post-calibration of the electrodes showed they retained their sensitivity after six hours of implantation; it was 22 ± 5 nA/ μ M, which is not statistically different than the pre-implantation sensitivity (26 ± 6 nA/ μ M, Student's t-test, $P = 0.59$, $n = 6$ electrodes). Additionally, the sensors retained their selectivity for dopamine over ascorbic acid when compared to the pre-calibration (post-implantation selectivity of 515 ± 180 , Student's t-test, $P = 0.95$, $n = 6$ electrodes). The post-implantation selectivity for dopamine over DOPAC for the coated electrode was also retrained, 35 ± 3 not statistically different than the pre-implantation numbers (Student's t-test, $n = 6$ electrodes, $P = 0.20$). This indicates that the electrode coating properties are not changed significantly during an acute *in vivo* measurement.

[0052] A NAFION® and PEDOT containing composite polymer has been electropolymerized in a novel scheme on carbon-fiber microelectrodes. The robust and reproducible coating was applied voltammetrically in a solution of EDOT, NAFION®, and acetonitrile. Coated electrodes showed increased electrochemical sensitivity (2-10x) and selectivity (10-30x), a comparable temporal response, lower noise, and good mechanical stability. Coated electrodes did not lose statistically significant selectivity and sensitivity after being implanted in the brain for 6 hours, which is an improvement over uncoated

electrodes. These data indicate that low-density PEDOT:NAFION® coated electrodes have a desirable combination of characteristics for various *in vivo* utilities, such as dopamine measurements. The low-density PEDOT:NAFION® coating appeared to be more well suited than the high-density PEDOT:NAFION® coating because it retained electrochemical information by preserving the voltammogram shape. However, in circumstances where high sensitivity is required, the high-density PEDOT:NAFION® coated electrodes may be more useful. The carbon fibers with composite material coating have shown to be useful in various *in vivo* measurements, such as *in vivo* dopamine measurements. It has also shown to be more advantageous for *in vivo* measurements of other neurotransmitters.

[0053] The foregoing discussion of the invention has been presented for purposes of illustration and description. The foregoing is not intended to limit the invention to the form or forms disclosed herein. Although the description of the invention has included description of one or more embodiments and certain variations and modifications, other variations and modifications are within the scope of the invention, e.g., as may be within the skill and knowledge of those in the art, after understanding the present disclosure. It is intended to obtain rights which include alternative embodiments to the extent permitted, including alternate, interchangeable and/or equivalent structures, functions, ranges or steps to those claimed, whether or not such alternate, interchangeable and/or equivalent structures, functions, ranges or steps are disclosed herein, and without intending to publicly dedicate any patentable subject matter. All references cited herein are incorporated by reference in their entirety.

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What is claimed is:

1. A carbon fiber comprising a substantially uniformly coated composite material, wherein said coated composite material comprises a sulfonated tetrafluoroethylene based ionomer.
2. The carbon fiber of Claim 1, wherein said sulfonated tetrafluoroethylene based ionomer comprises ethanesulfonyl fluoride, 2-[1-[difluoro-[(trifluoroethenyl)oxy]methyl]-1,2,2,2-tetrafluoroethoxy]-1,1,2,2-tetrafluoro-, with tetrafluoroethylene, tetrafluoroethylene-perfluoro-3,6-dioxo-4-methyl-7-octenesulfonic acid copolymer, or a mixture thereof.
3. The carbon fiber of Claim 1, wherein said composite material comprises NAFION® and a thiophene polymer.
4. The carbon fiber of Claim 3, wherein said thiophene polymer comprises poly(3,4-ethylenedioxythiophene (PEDOT)).
5. The carbon fiber of Claim 1, wherein said carbon fiber is configured for use as an electrode.
6. The carbon fiber of Claim 5, wherein said carbon fiber is configured for use as a microelectrode.
7. The carbon fiber of Claim 6, wherein the thickness of said coated composite material is about 100 nm.
8. The carbon fiber of Claim 1, wherein said carbon fiber has an increased sensitivity to an amine compound.
9. A method for producing a carbon fiber comprising a coating of a composite material, wherein said composite material comprises a sulfonated tetrafluoroethylene based ionomer, said method comprising:
 - placing a carbon fiber in a solution comprising a thiophene monomer and a sulfonated tetrafluoroethylene based ionomer in an organic solvent; and
 - applying electricity to said solution under conditions sufficient to produce a carbon fiber comprising a coating of a sulfonated tetrafluoroethylene based ionomer.
10. The method of Claim 9, wherein said composite material further comprises a thiophene polymer, wherein said thiophene polymer is derived from polymerization of said thiophene monomer.
11. The method of Claim 9, wherein said thiophene monomer comprises ethylenedioxythiophene (EDOT).
12. The method of Claim 9, wherein said composite material comprises said sulfonated tetrafluoroethylene based ionomer and a polymer of said thiophene monomer.

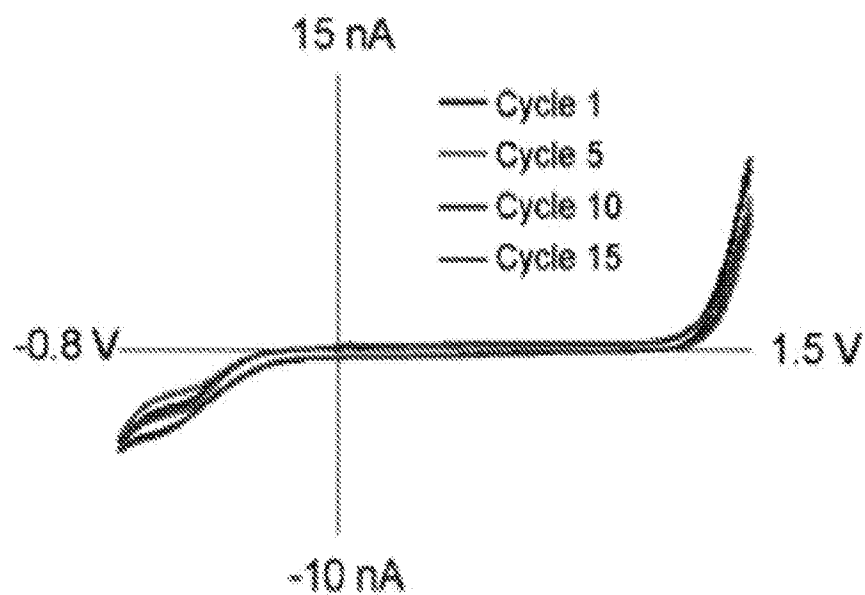


FIG. 1A

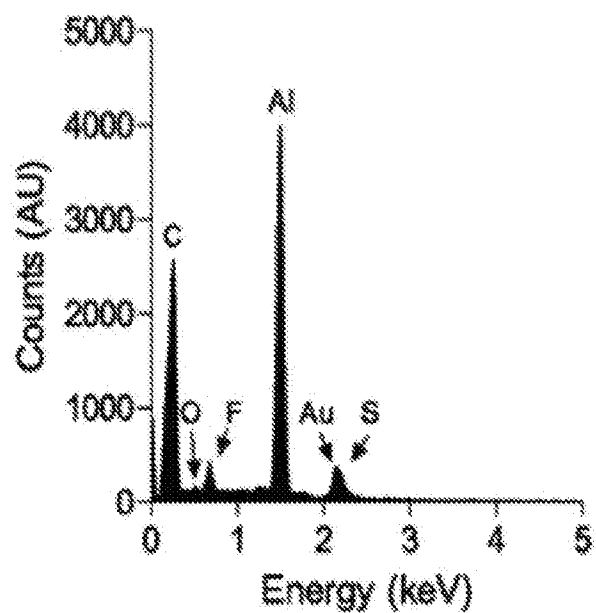


FIG. 1B

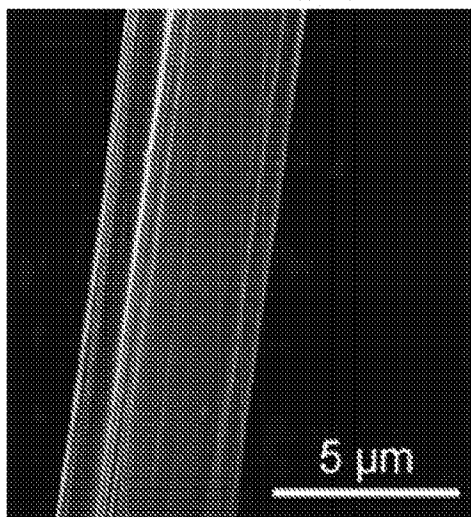


FIG. 1C

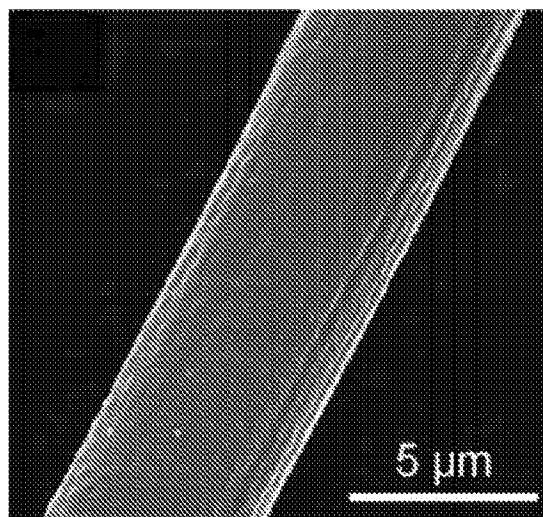


FIG 1D

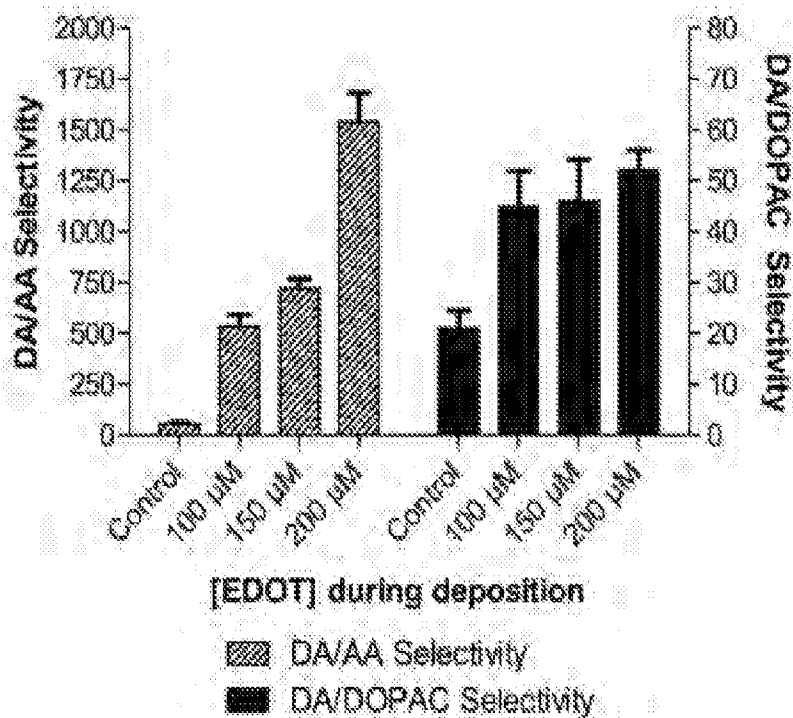


FIG. 4

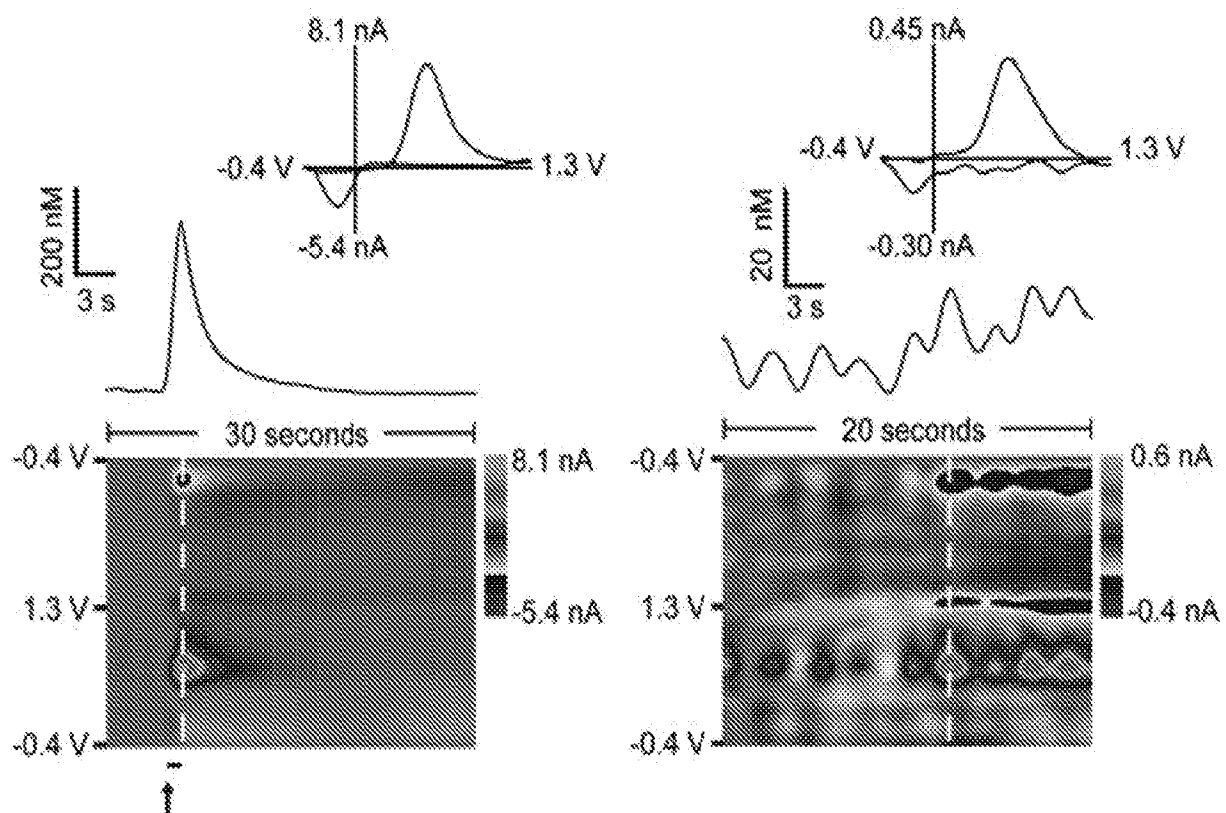


FIG. 6

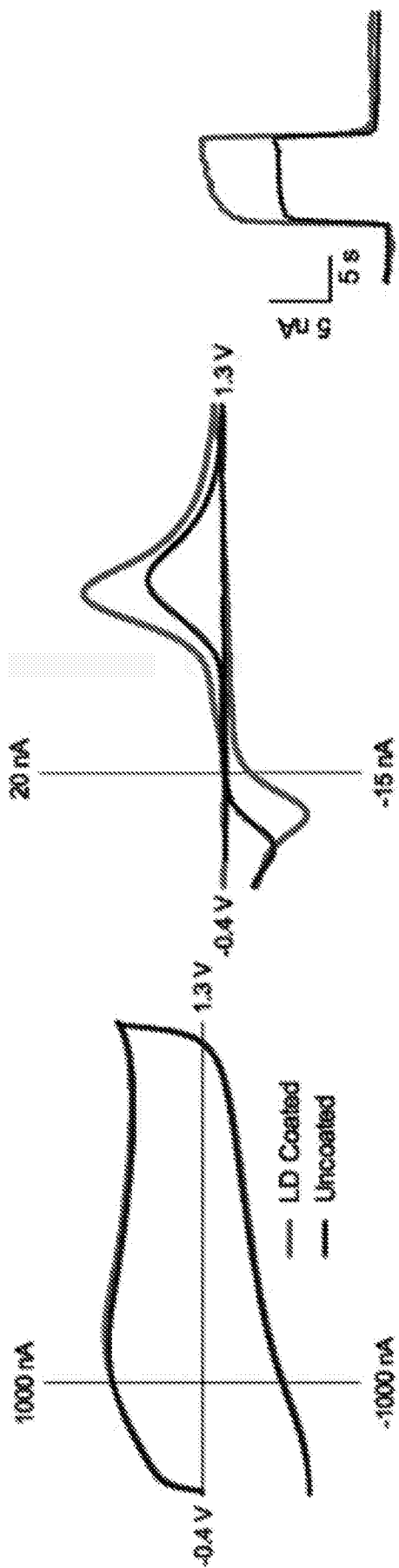


FIG. 2A

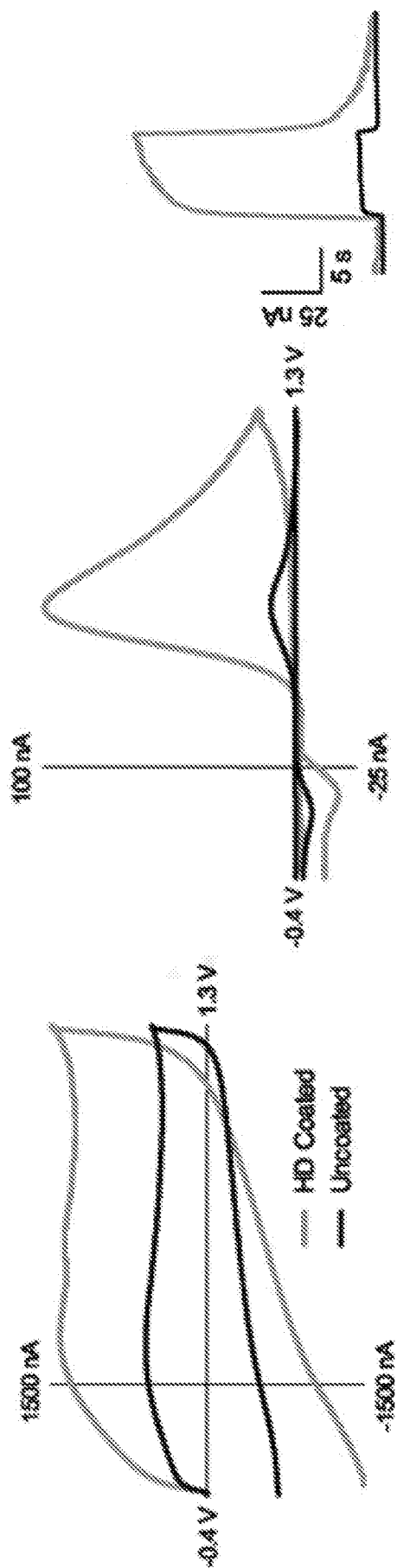


FIG. 2B

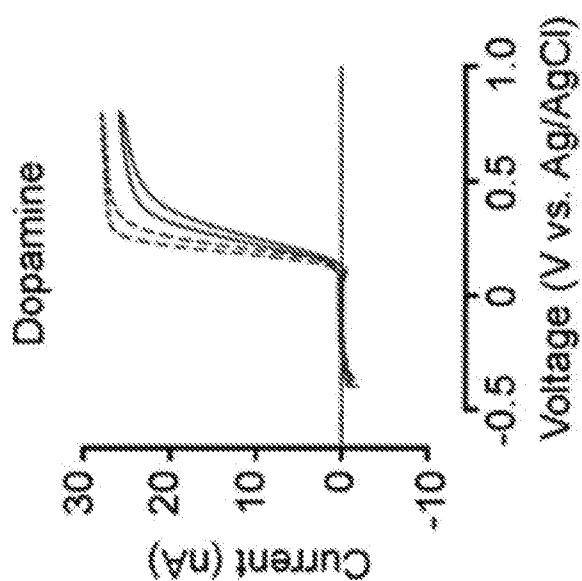


FIG. 3A

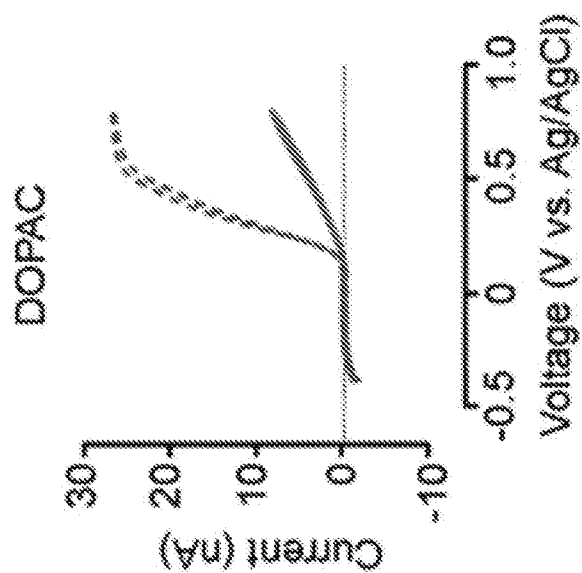


FIG. 3B

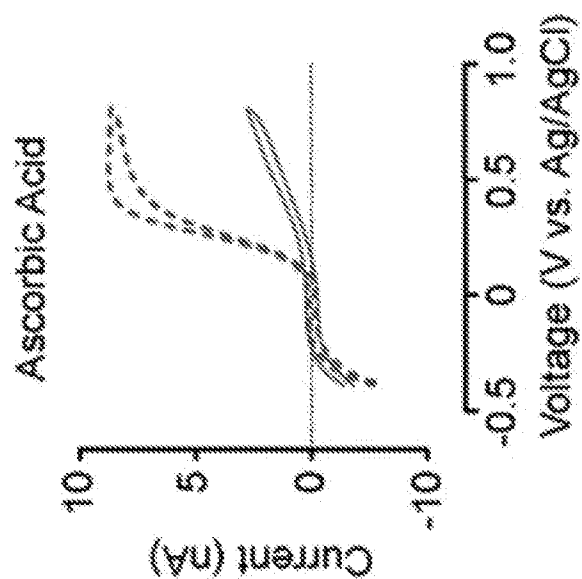


FIG. 3C

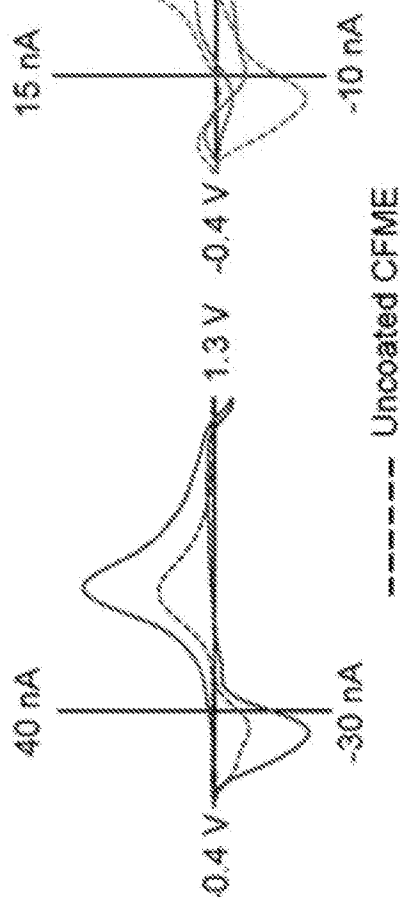


FIG. 3D

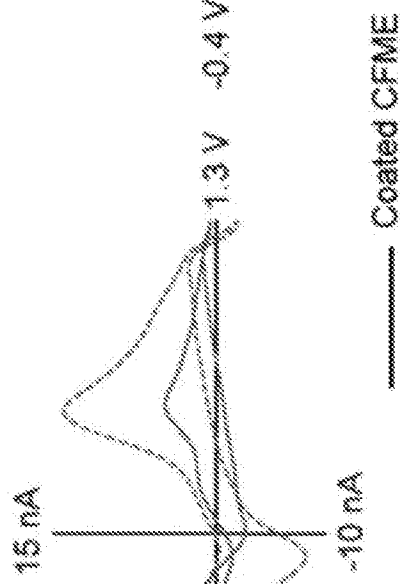


FIG. 3E

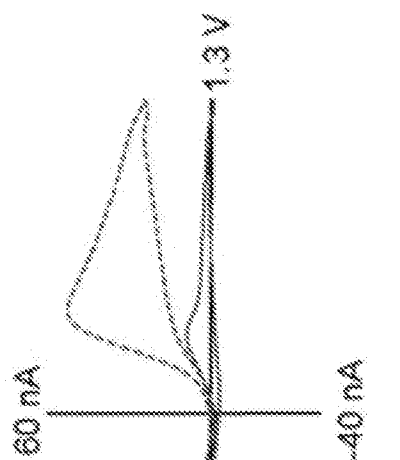


FIG. 3F

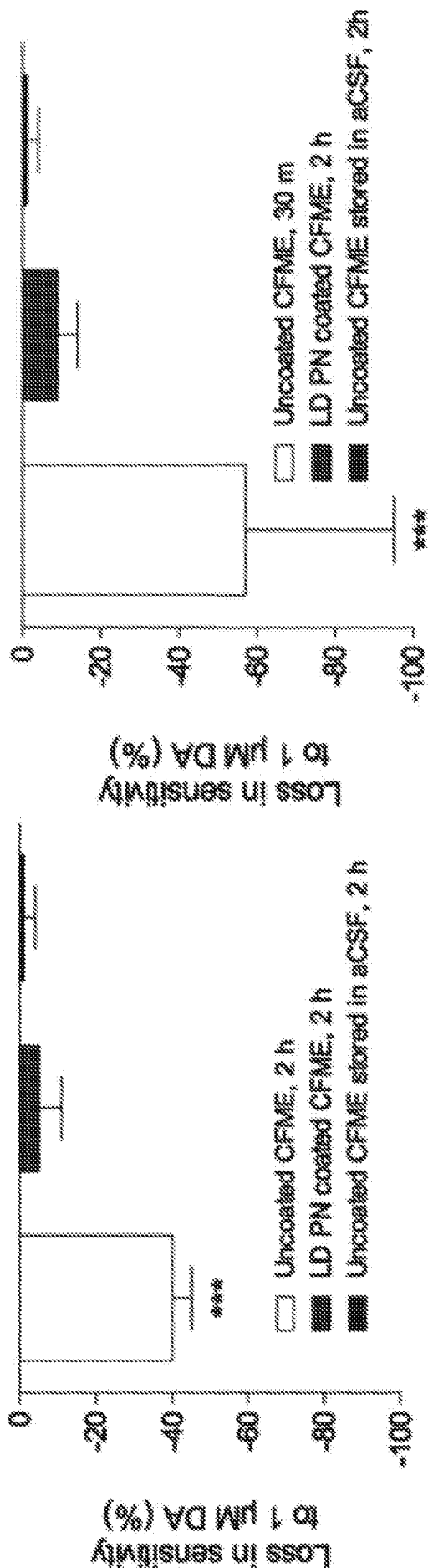


FIG. 5A

FIG. 5B

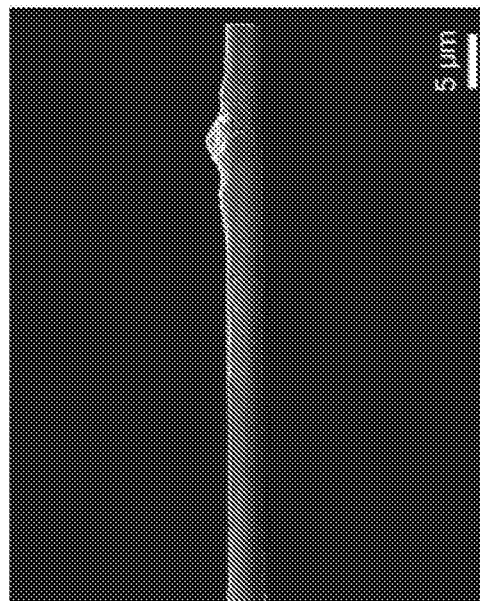


FIG. 5C

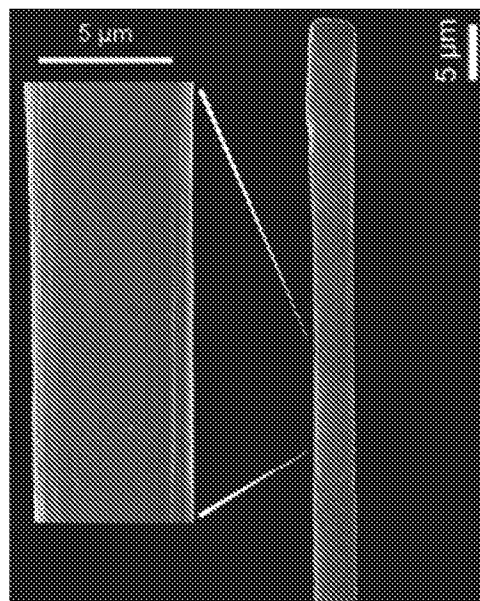


FIG. 5D

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/062814

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - H01M 4/04 (2016.01)

CPC - H01M 4/04 (2015.12)

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)

IPC(8) - H01B 1/20; H01M 2/14, 2/16, 4/02, 4/04 (2016.01)

CPC - H01B 1/20; H01M 2/14, 2/16, 4/02, 4/04 (2015.12)

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

USPC - 252/519.34; 427/222; 429/213; IPC(8) - H01B 1/20; H01M 2/14, 2/16, 4/02, 4/04; CPC - H01B 1/20; H01M 2/14, 2/16, 4/02, 4/04 (keyword delimited)

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

Orbit, Google Patents, Google Scholar.

Search terms used: carbon, fiber, thiophene, nafion, tetrafluoroethylene.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0020734 A1 (JANG et al) 22 January 2009 (22.01.2009) entire document	1-3, 5
---		-----
Y		4, 6-12
Y	US 2014/0272569 A1 (GM GLOBAL TECHNOLOGY OPERATIONS LLC) 18 September 2014 (18.09.2014) entire document	4, 6, 7
Y	US 8,394,330 B1 (LEWIS et al) 12 March 2013 (12.03.2013) entire document	8
Y	US 4,738,757 A (NAARMANN) 19 April 1988 (19.04.1988) entire document	9-12
P, X	VREEI AND et al. Biocompatible PEDOT:Nafion Composite Electrode Coatings for Selective Detection of Neurotransmitters in Vivo. Analytical Chemistry, Vol. 87, 18 February 2015, Pgs. 2600-2607.	1-12

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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"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

"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

"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

"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

"&" document member of the same patent family

Date of the actual completion of the international search

20 January 2016

Date of mailing of the international search report

05 FEB 2016

Name and mailing address of the ISA/

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