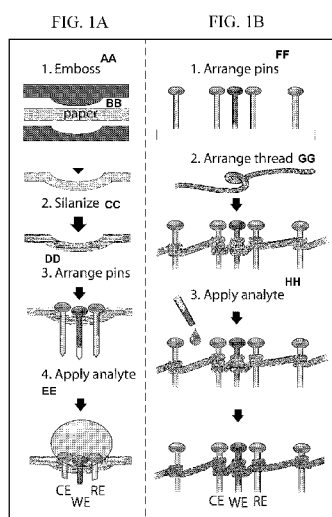




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[Continued on next page]

(54) Title: ELECTROANALYTICAL DEVICES WITH PINS AND THREAD



AA Estampage
BB Papier
CC Silanisation
DD, FF Agencement des broches
EE, HH Application d'analyte
GG Agencement du fil

(57) Abstract: An electroanalytical device includes a pin set comprising at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a piercing tip; and a hydrophobic or omniphobic paper substrate, wherein the substrate is shaped to provide at least one recess for holding a liquid, wherein the shafts of two conductive pins traverse the paper substrate to anchor the heads of the two conductive pins on the recess surface. An electroanalytical device can also include at least two conductive pins for use as working and counter electrodes, a thread, serially wound around the shafts of each of the two conductive pins; and a base into which the piercing tip of each of the pins is secured.



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ELECTROANALYTICAL DEVICES WITH PINS AND THREAD

RELATED APPLICATIONS

[0001] This application claims the benefit of priority under 35 U.S.C. §119(e) to United States Application Ser. No. 62/213,864, filed September 3, 2015, the contents of which is incorporated by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT

[0002] The present invention was made with United States government support under HDTRA Grant No. HDTRA1-14-C-0037 awarded by the Department of Defense. The United States government has certain rights in this invention.

TECHNICAL FIELD

[0003] This technology relates generally to diagnostics and electroanalytical devices. In particular, this invention relates to low cost analytical devices.

BACKGROUND

[0004] The most widely used methods for fabrication of electrodes in paper-based and thread-based electroanalytical devices (e.g. screen printing, stencil printing, gravure, chemical vapor deposition with shadow masking) require custom-patterned components such as screens, stencils or masks to deposit conductive materials on the surface of a substrate. The fabrication of these custom-patterned components is time-consuming, and can be expensive. In the finished device, the position of the electrode cannot be altered after the fabrication process has been completed.

[0005] Existing strategies for the fabrication of electrodes are particularly challenging on non-planar substrates such as embossed hydrophobic and omniphobic paper, and thread. The deposition of conductive materials on porous media such as paper or thread can alter its interfacial energy, porosity, tortuosity, and wicking. Inhomogeneity in the distribution of fluid can also affect the stability of the liquid electrode interface and influence the performance of the electroanalytical devices. These effects are most significant in the case of thread, where the fluid flow is confined to one dimension, and might be among the reasons

behind the few examples of electroanalytical devices that utilize thread (in contrast to the widespread interest in those that utilize paper).

[0006] Microfluidic paper-based electroanalytical devices (E μ PADs), have been used to detect a wide variety of analytes, including small-molecule metabolites, metal ions, nucleic acids, and serum proteins. E μ PADs use capillary-driven flow to transport aqueous solutions of analyte through the hydrophilic matrix of cellulose paper, or through “hollow” channels in hydrophilic paper to the surface of an integrated electrode. In E μ PADs and related systems, screen-printing is the most common method used for the fabrication of electrodes.

[0007] Other paper-based electroanalytical devices do not wick liquids through the cellulose matrix, but rather rest on the surface of electrodes printed on the surface of omniphobic or hydrophobic paper. The electrodes were printed on the surface of the embossed, silanized hydrophobic paper using inkjet printing or a pen-on-paper approach.

[0008] Thread-based microfluidic analytical devices (μ TADs) have been reported. Thread has been used as a matrix for bioassays with colorimetric detection and in the fabrication of electrochemical transistors. Very few thread-based analytical devices have so far been integrated with electrochemical detection. Sekar et al (Electrochem. Commun. 2014, 46, 128-131) describe one use of thread in voltammetric analysis; this demonstration more closely resembles a conventional electrochemical cell than a μ TAD. Individual pieces of thread were first coated with screen-printing pastes (carbon paste for the working and counter electrodes, and Ag/AgCl for the reference electrode), then coated with a thick layer of candle wax; one end of the thread was connected to a potentiostat, while the other end was immersed in a beaker containing a large volume (several milliliters) of a solution of analyte. Wei et al. (Microfluid. Nanofluid, 2013, 14, 583-590) also reported the use of a microfluidic chip based on thread for electrophoretic separation and detection of electroactive ions. The electrodes are fabricated by sequentially sputtering platinum and gold, using an aluminum mask, on protruding features hot embossed in a PMMA substrate.

[0009] Small-diameter metal electrodes—wires, microwires, needles, and hollow microcylinders—and non-metal fibers—graphite fibers or coated yarns—have been used as electrodes in a variety of electrochemical applications. Stainless steel has been used as a material for auxiliary and quasireference electrodes in electroanalytical flow systems and other electrochemical applications. Fernández-Abedul et al. (Anal. Chim. Acta, 1990, 237,

127-133) reported a flow system in which a hollow stainless-steel cylinder acted as both the outlet of the flow and the auxiliary electrode. Wojciechowski et al. (Anal. Chim. Acta, 1996, 328, 67-71) used stainless-steel syringe needles as reference and auxiliary electrodes for anodic stripping voltammetry.

SUMMARY

[0010] A new class of electrodes for thread-based or hydrophobic or omniphobic paper-based analytical systems is described. Prefabricated stainless steel pins—either unmodified, or coated with a thin layer of graphite ink— provides a simple solution to the problems of fabrication and integration of electrodes in a low-cost analytical device. Pins are used as electrodes in systems fabricated using either omniphobic or hydrophobic paper or thread. As electrodes, pins are sensitive and can be used to quantify metabolites. Surprisingly, because they offer readily accessible connection points to electrochemical readers and easily modifiable configurations, pin electrodes allow the fabrication of devices suitable for multiplexed analysis. Thread-based arrays are provided that can be used to detect different analytes in the same array, or to perform multiple measurements of the same analyte simultaneously, or in close succession. Devices having 96-well plates in omniphobic paper, that is chemically treated to be omniphobic, can be used to perform independent measurements in each well.

[0011] In one aspect, an electroanalytical device includes a pin set comprising at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a piercing tip; and a hydrophobic or omniphobic paper substrate, wherein the substrate is shaped to provide at least one recess for holding a liquid, wherein the shafts of two conductive pins traverse the paper substrate to anchor the heads of the two conductive pins in electrical contact with the recess surface.

[0012] In one or more embodiments, the pin set further includes a third conductive pin for use as a reference electrode, wherein the third pin is comprised of a head, a shaft and a piercing tip and wherein the shaft of the third pin traverses the paper substrate to anchor the head of the third conductive pin on the well surface.

[0013] In any of the embodiments, the pins comprise stainless steel pins.

[0014] In any of the embodiments, the conductive pin comprises a conductive coating.

[0015] In any of the embodiments, the working electrode conductive pin comprises a conductive coating.

[0016] In any of the embodiments, the coating is selected from the group consisting of conductive carbon.

[0017] In any of the embodiments, the carbon coating is selected from the group consisting of graphite and carbon nanotubes and mixtures thereof

[0018] In any of the embodiments, the hydrophobic or omniphobic paper substrate is chemically functionalized with hydrophobic or omniphobic functional moieties.

[0019] In any of the embodiments, the recess is embossed into the paper substrate.

[0020] In any of the embodiments, the recess is a well or a channel.

[0021] In any of the embodiments, the paper substrate comprises a plurality of recesses.

[0022] In any of the embodiments, each of the recesses contains a pin set.

[0023] In any of the embodiments, the device is a 96 well device.

[0024] In any of the embodiments, the device further includes one or more reagents located in the recess, wherein the reagents are selected to interact with an analyte of interest.

[0025] In another aspect, a method of making an electroanalytical device includes providing at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a piercing tip; and inserting each of the pins into a recess in a hydrophobic or omniphobic paper substrate, and locating the pin head in the recess of the paper substrate.

[0026] In another aspect, a method of electroanalysis includes providing an electroanalytical device as described herein; introducing a liquid into the recess of the device; and reading out an electrical signal, said signal indicating a property or state of the liquid analyte.

[0027] In another aspect, an electroanalytical device includes a pin set comprising at least two conductive pins for use as working and counter electrodes, wherein the first and second

pins are comprised of a head, a shaft and a piercing tip; a thread, serially wound around the shafts of each of the two conductive pins; and a base into which the piercing tip of each of the pins is secured.

[0028] In any of the embodiments, the pin set further comprises a third conductive pin for use as a reference electrode, wherein the third pin is comprised of a head, a shaft and a piercing tip and wherein the shaft of the third pin traverses the paper substrate to anchor the head of the third conductive pin on the well surface.

[0029] In any of the embodiments, the pins comprise stainless steel pins.

[0030] In any of the embodiments, the conductive pin comprises a conductive coating.

[0031] In any of the embodiments, the working electrode conductive pin comprises a conductive coating.

[0032] In any of the embodiments, the coating is selected from the group consisting of conductive carbon.

[0033] In any of the embodiments, the carbon coating is selected from the group consisting of graphite and carbon nanotubes and mixtures thereof

[0034] In any of the embodiments, the device includes a plurality of conductive pin sets, and the thread is wound in series around each of the pin sets.

[0035] In any of the embodiments, the device further includes a barrier located on the thread between pin sets.

[0036] In any of the embodiments, the device includes a plurality of conductive pin sets, and a plurality of thread and each thread is wound around one pin set.

[0037] In another aspect, a method of making an electroanalytical device includes providing at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a piercing tip; inserting each of the pins into a base; and serially winding a thread around the shafts of each of the two conductive pins.

[0038] In another aspect, a method of electroanalysis includes providing an electroanalytical device as described herein; and introducing a liquid the thread of the device; and reading out an electrical signal, said signal indicating a property or state of the liquid analyte.

[0039] Embodiments can be variously combined or separated without parting from the invention.

[0040] In one aspect, the invention provides low-cost electroanalytical devices that can be to assemble and reconfigures “on-the fly” to meet the needs of specific applications and settings. These embodiments illustrate electrodes that are by themselves nearly as ubiquitous, portable, inexpensive, and easily storable as are paper and thread. The combination of stainless-steel pins—untreated or coated with a thin layer of carbon ink—and embossed omniphobic paper or cotton thread, provides the basis for the fabrication of simple, versatile and low-cost electroanalytical devices. These devices are inexpensive and lightweight, exhibit diffusion-controlled electrochemical behaviors, and can be used with biological samples. The paper and thread-based devices fabricated using this method have the potential to provide new functional options in clinical diagnostics, environmental monitoring, and microfluidic and electronic systems.

[0041] These and other aspects and embodiments of the disclosure are illustrated and described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0042] The invention is described with reference to the following figures, which are presented for the purpose of illustration only and are not intended to be limiting.

In the Drawings:

[0043] Figure 1A is a schematic representation of a process used for the fabrication of electrochemical cells in embossed omniphobic paper, according to one or more embodiments; and Figure 1B is a schematic representation of the process used for the fabrication of an electrochemical cell with cotton thread, in one or more embodiments in which cases stainless steel pins are used as reference and counter electrodes (RE and CE), and a stainless steel pin coated with a graphite and carbon nanotube ink is used as the working electrode (WE).

[0044] Figure 2A shows top (A1) and side (A2) photographs of an electrochemical cell fabricated using embossed omniphobic paper and stainless steel pins as reference and counter electrodes (RE and CE), and a stainless steel pin coated with a graphite and carbon nanotube ink as working electrode (WE), in which the electrodes are placed at a distance of ~ 0.1 inch (2.53 mm) away from one another.

[0045] Figure 2B shows cyclic voltammograms recorded in a 500 μM solution of FcCO_2H in 1x PBS, pH 7.6 at a scan rate of 100 mVs^{-1} for seven independent embossed omniphobic paper devices, prepared as illustrated in Figure 2A.

[0046] Figure 2C is a photograph of an electrochemical cell fabricated using cotton thread and stainless steel pins as reference and counter electrodes, and a stainless steel pin coated with a graphite and carbon nanotube ink as working electrode, in which the electrodes are placed at a distance of ~ 0.1 inch (2.53 mm) away from one another.

[0047] Figure 2D shows cyclic voltammograms recorded in a 500 μM solution of FcCO_2H in 1x PBS, pH 7.6 at a scan rate of 100 mVs^{-1} for seven independent thread-and-pin arrays prepared as illustrated in Figure 2C.

[0048] Figures 3A and 3B show cyclic voltammograms of 500 μM FcCO_2H in 1x PBS (pH 7.6) in electrochemical cells fabricated using (3A) embossed omniphobic paper and (3B) thread, at various scan rates ascending along y-axis: 10, 20, 50, 100, 200, 300 mV s^{-1} .

[0049] Figure 3C shows the plot of anodic and cathodic peak currents vs. the square root of the scan rate ($v^{1/2}$) conducted on an omniphobic paper device (up-pointing triangle : anodic peak current, down-pointing triangle : cathodic peak current) and in a thread device (circles : anodic peak current, squares : cathodic peak current), in which the dashed lines represent linear regressions with equations: $y_1 = 6 \times \mu\text{AV}^{-1/2} \text{ s}^{1/2} + 0.1 \mu\text{A}$ [$R^2 = 0.991$], $y_2 = 5 \times \mu\text{AV}^{-1/2} \text{ s}^{1/2} - 0.05 \mu\text{A}$ [$R^2 = 0.986$], $y_3 = -4 \times \mu\text{AV}^{-1/2} \text{ s}^{1/2} + 0.05 \mu\text{A}$ [$R^2 = 0.989$], $y_4 = -6 \times \mu\text{AV}^{-1/2} \text{ s}^{1/2} - 0.05 \mu\text{A}$ [$R^2 = 0.992$] and error bars correspond to standard deviation of measurements performed using seven independent devices.

[0050] Figure 4A shows chronoamperograms recorded on thread for the mixture of the enzymatic assay for the determination of lactate, at concentrations between 1.1-20 mM; the chronoamperograms were recorded at 0.4 V *versus* a stainless steel quasi-reference pin electrode.

[0051] Figure 4B shows the calibration plots of the currents recorded after 40s as a function of concentration of lactate on thread-and-pins arrays and in wells embossed in omniphobic paper, in which the dashed black line represents a fit to the equation: $y=0.08x+1.13$ ($R^2=0.994$) whereas the red line represents a fit to the equation: $y=0.06x+0.91$ ($R^2=0.987$), for concentrations of lactate between 1.1-20 mM and error bars correspond to standard deviation of measurements performed using 7 independent devices.

[0052] Figures 5A and 5B are photographs of a 96 well plate fabricated using embossed omniphobic paper and pin electrodes (5A) before and (5B) after 50 μ L drops of an aqueous solution are added to each well.

[0053] Figures 5C and 5D shows independent voltammograms recorded in different wells a1 and a2 of the plate of Figures 5A and 5B, respectively, in which a 100 μ M solution of hydroquinone (HQ), and a 250 μ M solution of FCA, both in 1x PBS, pH 7.6, is recorded at a scan rate of 100 mV s⁻¹; the presence of a different analyte in a neighboring well does not interfere with measurements.

[0054] Figure 6A is schematic and Figure 6B is a photograph of a device comprising multiple alternating stainless steel pins and carbon-coated stainless steel pins and a single thread that forms three helical turns around each pin; the device can be used for multiple measurements (in rapid succession) of the same analyte on thread.

[0055] Figure 6C shows chronoamperograms at 0.4 V recorded with each of the seven cells along the thread, using in each case the two adjacent stainless steel pins as CE and RE. Solution: 100 mM potassium ferricyanide in 1x PBS pH 7.6.

[0056] Figure 7A is a schematic and Figure 7B a photograph of a device comprising three distinct electrochemical cells formed from a single thread; arrows indicate the presence of a small amount of polymer (cyanoacrylate) that serves as a boundary between consecutive cells. The device can be used for measurement of three different analytes (in rapid succession or simultaneously).

[0057] Figure 7C is a square-wave voltammograms recorded with each electrochemical cell, for: (1) left: 10 μ M FCA in PBS, pH 7.6; (2) middle: buffer only, PBS, pH 7.6; (3) right: 100 μ M FCA in PBS, pH 7.6, the center cell, where the thread is wet with buffer only, has no observable interference with neighboring cells.

DETAILED DESCRIPTION

[0058] Devices using conductive pins and paper and thread as a substrate for the fabrication of the electrochemical cells are described. Paper and thread are widely available, inexpensive, lightweight, and flexible.

[0059] In one aspect, an electroanalytical device employing conductive pins is described. The electroanalytical device can include at least two conductive pins for use as working and counter electrodes. Optionally, the electroanalytical device includes a third pin or more pins that can function as a reference electrode(s). The device can also include multiple pin sets that serve as multiple cells in the device. The conductive pins can be straight pins having head, a shaft and a piercing tip. In one or more embodiments, the conductive pin is a stainless steel pin; however, other low corrosive metal pins, such as brass pins and the like, can be used. Stainless-steel pins have several characteristics that make them attractive as candidates for adaptive use as electrodes in electrochemical devices. Stainless steel pins are inexpensive (less than \$0.001/per pin when purchased from commercial retailers, and much less if purchased wholesale) and available nearly all over the globe. Stainless steel is highly conductive and stable electrochemically in neutral or mildly acidic or basic aqueous solutions.

[0060] In one or more embodiments, the conductive pins can be coated. The coating can be used to generate an electroactive surface area of the working electrode that is sufficiently large to be useful for analysis. In one or more embodiments, the conductive pins can be coated with a conductive carbon powder. For example, the stainless-steel pin can be coated with carbon ink prepared by mixing graphite paste and solvent thinner with a multi-walled carbon nanotube powder.

[0061] In one or more embodiments, the conductive pins are used as electrodes on a paper substrate. The paper can be any woven or non-woven cellulosic substrate. The paper substrate can be a hydrophobic or omniphobic paper substrate (also referred to herein as R_F paper), where the substrate is shaped to provide at least one well for holding a liquid. Paper can be rendered hydrophobic or omniphobic by chemically functionalizing with hydrophobic or omniphobic functional moieties. The paper can be shaped by embossing and rendered omniphobic using a gas-phase treatment with a fluorinated organosilane. The omniphobic or hydrophobic properties of the paper allow an aqueous liquid to sit above the surface of the

paper. That is, the liquid is not wicked into the paper. Details on the manufacture of omniphobic or hydrophobic paper can be found in Glavan et al., *Adv. Funct. Mater.*, 2014, 24, 60-70, which is incorporated by reference.

[0062] To complete the device, the shafts of two conductive pins traverse the paper substrate to anchor the heads of the two conductive pins on or near the well surface. The pin heads therefore sit in the well and are wetted by a liquid solution that is applied to the well. The shafts of the pins can be used to make the electrical connection, e.g., to a potentiostat, used to enable the electrodes to function.

[0063] Different parts of a pin (the head, the shaft and the sharp tip) can be used for different purposes: the head can serve as an electrode in omniphobic paper-based devices, part of the stem can serve as an electrode in thread-based devices, the stem can be used for connection to the potentiostat, and the sharp tip can be used to anchor the pins in a mechanical support.

[0064] Figure 1A illustrates the manufacture of an electroanalytical device employing paper and pins, in which the pins serve as working electrode (WE), reference electrode (RE), and counter electrode (CE) according to one or more embodiments. As shown in Figure 1A, panel 1, paper is first embossed to provide a desired shape – as shown here by example a well shape. However, other shapes, such as channels and the like are also contemplated. After embossing, the paper is rendered hydrophobic or omniphobic, e.g. by silanizing using fluoroalkyl trichlorosilanes, as illustrated in Figure 1A, panel 2.

[0065] Next the pins serving as working electrode (WE), reference electrode (RE), and counter electrode (CE) are inserted in an embossed well. The pins can be cleaned by sonication in isopropyl alcohol, e.g., for 20 min, and used without further modification as reference and counter electrodes. The working electrode (central pin in Figure 1A, panel 3, is coated with a conductive carbon layer. The working electrode was a stainless-steel pin coated with freshly prepared carbon ink. The carbon ink can be prepared using graphite ink (e.g., C10903P14, from Gwent Electronic Materials Ltd, Montypool, UK), multi-walled carbon nanotube powder (e.g., # 724769, from Sigma-Aldrich) and ET160 solvent thinner (Ercon Inc., Wareham, MA). These components are mixed in mass ratio 49.9 % : 0.2 % : 49.9 %, respectively. The mixture can be sonicated, e.g., for 15 min, using a high power tip sonicator (Branson Sonifier 450, with a Micro 3/16 tip), with 50% duty cycle

at 50% power (total power 400W). This procedure provides homogeneous ink, with no particles or phase separation.

[0066] The stainless steel pins can be immersed in the solution, removed and allowed to dry, e.g., for 5 min, at room temperature, then dried, e.g., for 5 min, in an oven, e.g., at 110° C. The process can be repeated, e.g., 3 times, resulting in a coating thickness around the shaft of the pin of ~30 µm, and around the head of the pin ~100 µm.

[0067] The electrodes are placed spaced apart, e.g., 0.1 in (~2.53 mm) away, from one another in the embossed wells, using a transparency with precut holes as an alignment tool.

[0068] A liquid analyte added to the device rests on the surface of the well, and forms an interface with the surfaces of the heads of the pins, as illustrated in Figure 1A, panel 4. To allow the solution of analyte to contact the electrodes, a micropipette (or any other liquid application method) can be used to add a drop of liquid to the embossed omniphobic well. The approximate geometrical area of the interface in this exemplary embodiment is 5 mm². Figure 2A A1 shows a top view of a 3-pin electrochemical device in embossed paper and Figure 2A A2 shows a side view of the same device with an applied liquid analyte.

[0069] In other embodiments, an absorbent thread can be used as the substrate for the electroanalytical device. In one or more embodiments, the thread is a cotton thread, although, any hydrophilic, absorbent thread could be used. In one or more embodiments, the thread can be treated, such as plasma treatment, to increase the hydrophilicity of the thread.

[0070] As noted in the previous embodiments, the electroanalytical device can include at least two conductive pins for use as working and counter electrodes. Optionally, the electroanalytical device includes a third pin that can function as a reference electrode. Electrical contact with an analyte is accomplished by winding a thread around each of the pin shafts that serve as the working electrode (WE), reference electrode (RE), and counter electrode (CE). Any number of windings are contemplated and the winding is selected to provide sufficient physical and electrical contact between the thread and the pin electrodes. The pins are positioned and arranged so that the thread serially contacts first the counter electrode and then the working electrode and then the optional reference electrode. Terminally located pins can be used to define application and detection zones. The pins and the spanning thread can be supported by sticking the pins into a supporting base. On

application of a liquid to the thread, the liquid wicks along the thread and forms a cylindrical interface with the shaft of each pin, which can provide an electrochemical readout.

[0071] Figure 1B shows the design of an electrochemical cell, in which pins (WE, RE, CE) are surrounded by helical turns of thread according to one or more embodiments. The number of windings around the pin shaft can be used to control the contact area. The pins can be cleaned and the working electrode can be coated as described above. Cotton thread (e.g., YLI Fiberactive Organic Cotton Thread, 24/3 ply TEX 60) can be washed with a solution, e.g., of 0.05% Span 60, rinsed and air dried, then plasma oxidized, e.g., for 30 min, to increase its hydrophilicity.

[0072] As shown in Figure 1B, panel 1, 5 pins are secured to a solid base. The plastic, empty housing of a 2.53 mm male PCB single row strip connector was used to guide the insertion of the pins in a mechanical support (here, PDMS) and maintain constant spacing between them. The terminal pins define the location of a single test zone and the central three pins serve as the counter, working and reference electrodes.

[0073] Once secured, a thread is wound around each pin as shown in Figure 1B, panel 2. For the formation of a three electrode array, ~70 mm-long pieces of thread can be used, for example. To control the tension in the thread, two knots can be placed, e.g., 1.5 inch (~38 mm) apart from each other. After the first knot is fixed with a support stainless steel pin, the thread can be sequentially wrapped around each electrode: two helical turns around the RE pin (apparent contact area 3 mm^2), 3 helical turns around the WE pin (apparent contact area 4 mm^2), 3 helical turns around the CE pin (apparent contact area 4 mm^2). After the array is complete, the second knot can be fixed with a support stainless steel pin.

[0074] Next, a liquid analyte is applied to an “application zone” of the device, as shown in Figure 1B, panel 3. To allow the solution of analyte to contact the electrodes, a micropipette (or any other liquid application method) can be used to add a drop of liquid either to the thread. In this embodiment, liquid contact with the shaft provides the electrical communication needed for analysis (as compared to the embodiment using paper, in which the head provided the contact with the liquid analyte). On the thread, the liquid wicks along the thread and forms a cylindrical interface with the shaft of each pin (Figure 2C). The approximate geometrical area of the interface is 4 mm^2 . Figure 2C shows a side view of an assembled string electrochemical device.

[0075] The performance of the pins as electrodes in thread-based and omniphobic RF paper based electrochemical cells can be evaluated by cyclic voltammetry (CV), chronoamperometry (CA), and square-wave voltammetry (SWV) with our electrochemical device using a commercial potentiostat (Autolab PGSTAT12, Metrohm). The paper device is connected to the potentiostat by anchoring a mini test clip leads, (x100w mini hook to banana socket, TestPath.com), to the stem of each pin electrode. All the electrochemical measurements were carried out at room temperature (23 ± 2 °C). To evaluate the performance of the electrodes, we used cyclic voltammetry (CV). This analysis is performed using cyclic voltammetry using a solution of FCA ($100 \mu\text{M}$ in PBS, pH 7.6) at different scan rates (10, 20, 50, 100, 200, and 300 mV s^{-1}). For SWV, the parameters were: scan potential between -0.4 V and 0.2 V ; amplitude= 0.10 V ; square wave frequency = 25 Hz ; step height = 0.005 V .

[0076] The cyclic voltammograms (CVs) were recorded at a scan rate of 100 mV s^{-1} of a solution of a redox probe with well-characterized electrochemical behavior (ferrocene carboxylic acid, FcCO_2H). Both electrochemical devices are able to produce reliable electrochemical data. Figures 2B and 2D show the variation in the CVs of the solution of FcCO_2H (at $100 \mu\text{M}$ in $1\times \text{PBS}$, pH 7.6) for paper-based and thread-based cells, each recorded using seven different devices. The device-to-device variation in the performance of the electrodes was, in both cases, less than 10% as indicated by the relative standard deviation (RSD, defined as the percentage ratio of the standard deviation to the mean of the distribution) of 6.3 % ($\sigma = 2.2 \mu\text{A}$) and 9.4 % ($\sigma = 1.6 \mu\text{A}$) in the anodic peak current, i_{pa} , for paper and thread-based cells, respectively.

[0077] To determine whether the electrochemical processes at the pin electrode-liquid interface in RF paper and thread-based devices are diffusion-controlled, cyclic voltammograms were recorded of $500 \mu\text{mol L}^{-1} \text{FcCO}_2\text{H}$ in PBS, pH 7.6, at scan rates between 10 and 300 mV s^{-1} . The anodic and cathodic peak currents (i_{pa} and i_{pc}) were linearly proportional to the square root of the scan rate in both RF paper ($R_2=0.991$ and 0.992 , respectively) and thread-based cells ($R_2=0.986$ and 0.989 , respectively) (see Figure 3C). These results indicate that, in both cases, the rate of the electrochemical reaction at the surface of the pin electrode is governed by the diffusion of FcCO_2H to the surface of the electrode.

[0078] These results are in agreement with results of finite-element simulations confirming that, for short diffusion distances and high scan rates, the geometry of the electrode (cylindrical or hemispherical, depending on whether the shaft or the head of the pin forms an interface with the solution of analyte) does not influence the process. Thus, under the given experimental conditions, the electrochemical cells fabricated using pin electrodes and either thread or RF paper can reproduce the classical diffusion limited electrochemical processes reported in EμPADs incorporating screen-printed electrodes.

Finite-element simulation

[0079] The finite element simulation was performed on COMSOL Multiphysics 4.1 (COMSOL AB, Stockholm, Sweden). The model considered one-dimensional time dependent convection diffusion for two species (C_R and C_O , corresponding to reduced and oxidized state of the mediator respectively). The electrochemical process was described with a reaction term at one of the boundaries converting C_R to C_O and the reverse, with a reaction rate determined by the current i . In eq. 3, n is the number of electrons transferred per reaction ($n=1$ for FcCO_2H), F is Faraday's constant, A is electrode area (here 5 mm^2), k is electron transfer efficiency (here assumed to be 1 cm s^{-1}), E^O is oxidation potential of for FcCO_2H (0.29V), α is the symmetry parameter of the reaction (0.5 for symmetric reactions, as assumed here) and E is the potential, scanned at a rate 100 mVs^{-1} , completing one full cycle with nodes 0V , $+0.5\text{V}$, -0.1V and 0V . For simplicity, the convection and migration terms were neglected; the diffusion constant was assumed to be $1.7 \cdot 10^{-10} \text{ m}^2 \text{ s}^{-1}$.

$$i(t) = nFAk \left(C_O(0, t)e^{-\alpha nF(E-E^O)/RT} - C_R(0, t)e^{(1-\alpha)nF(E-E^O)/RT} \right) \quad (3)$$

[0080] Mass transport occurs over less than $100 \mu\text{m}$, a small distance compared to the diameter of the pin (about $550 \mu\text{m}$). Therefore we can neglect the curved geometry of the pin and consider it equal to planar electrode described by abovementioned 1D model. Simulation reproduced the shape of voltammogram, as well as peak current ($\sim 2 \mu\text{A}$), but experimental peaks appeared at slightly lower voltages than in the simulation (0.27 V and 0.33 V in simulation, vs. 0.2 V and 0.27 V in experiments), while the peak potential difference is the same and close to theoretical expectation of 59 mV .

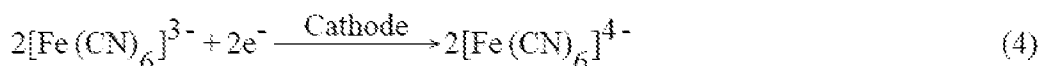
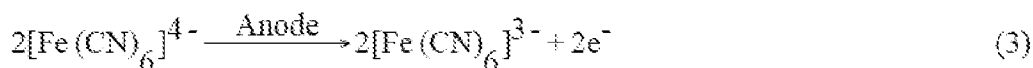
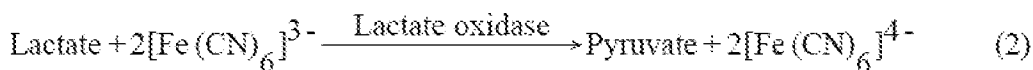
Applications in clinical diagnostics: analysis of L-lactate in human serum

[0081] The feasibility of using RF paper-based and thread-based devices to measure the concentration of a clinically relevant analyte, L-lactate, in human serum was evaluated. The range of L-lactate concentrations relevant for diagnosis is between 0.5 and 15–20 mM in serum. For the measurement of L-lactate, we mixed a solution of L-lactate in human plasma with a solution containing lactate oxidase and ferricyanide in a centrifuge tube. We prepared solution A containing 200 units/mL of lactate oxidase and 250 mM potassium ferricyanide in PBS buffer (pH 7.6). Solution B was prepared by diluting a 100-mM lactate standard (Biovision, Lactate Assay kit) in human serum (Innovative Research, Inc.; the plasma as received contained 1.1 mM L-lactate). A 45-μL volume of solution A was then mixed with 5 μL of solution B, and the reaction was allowed to proceed for 60 s. The solution was then applied either to an embossed well or a threaded array using a micropipette.

[0082] Chronoamperometry can be used to perform this demonstration of principle because it is a simple and frequently used technique that provides a quantitative result. Cyclic voltammetry (CV) is less useful for accurate quantitation of electroactive species than chronoamperometric or pulse voltammetric techniques, because the correction for the capacitive current in CV is typically ambiguous. Chronoamperometry measures current as a function of time at constant applied voltages, and starts with a large capacitive current that decays within the first few seconds. Faradaic current, which is proportional to the concentration of the analyte, becomes dominant, and decays according to the Cottrell equation (eq 1), where n is the number of electrons, F is Faraday's constant, A is the area of the electrode, D is the diffusion coefficient of analyte, C is the concentration of analyte, and t is time.

$$i = \frac{nFAD^{1/2}C}{\pi^{1/2}t^{1/2}} \quad (1)$$

[0083] In the lactate assay, potassium ferricyanide, $K_3[Fe(CN)_6]$, served as a mediator (eq 2-4):



[0084] For each mole of lactate that is oxidized, two moles of $[\text{Fe}(\text{CN})_6]^{3-}$ are reduced to $[\text{Fe}(\text{CN})_6]^{4-}$; the latter can be quantified using chronoamperometry at an applied voltage of 0.4 V vs. a stainless-steel quasi-reference electrode.

[0085] Figure 4A shows the calibration curves for the measurement of L-lactate, for values between 1.1 mM (the value initially present in the serum) and 20 mM (with additional lactate spiked in the serum). The sensitivity is 0.08 $\mu\text{A mM}^{-1}$ for the RF paper- and 0.06 $\mu\text{A mM}^{-1}$ for the thread-based device. Figure 4B shows the calibration plots of the currents recorded after 40s as a function of concentration of lactate on thread-and-pins arrays and in wells embossed in omniphobic paper.

Approaches to multiplexing: Electrochemical 96 well plate.

[0086] Embossing can be used to shape the paper into a microplate because it is simple, fast and requires simple equipment (only reusable molds generated easily using a 3D printer, printed molds costing $\sim \$0.32$ per gram of material, or about \$8 for a mold used to emboss the 96-well plate).

[0087] Paper can be rendered hydrophobic using a fast (five minute in the process we use), vapor-phase treatment with organosilanes. The treatment with 3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl trichlorosilane ($\text{CF}_3(\text{CF}_2)_7\text{CH}_2\text{CH}_2\text{SiCl}_3$, “ C_{10}^{F} ”) transformed paper in an omniphobic substrate.^[43] To keep the pins in stable position, we used a 1-mm thick paper (Whatman Gel Blot paper, GE Healthcare).

[0088] The diameter of the head of the pin can be approximated to be an oblate spheroid with diameter 1.5 mm and height 0.68 mm for the uncoated pin, and 1.5 mm and 0.77 mm for coated pin, respectively. From these parameters, we estimate that the macroscopic surface area of the WE in an omniphobic R^{F} -paper electrochemical cell is $\sim 5 \text{ mm}^2$.

[0089] Pins can be inserted in wells embossed in omniphobic paper such that the heads of the WE, CE, and RE pin were equally spaced, e.g., with a 0.1 in (2.53 mm) distance among them. A transparency sheet with precut holes can serve as alignment tool for the insertion of the pins. Single devices were separated from the 96-well plate by cutting, or individual wells were used while part of the intact 96-well plate.

[0090] A 96-well plate is capable of carrying out parallel analyses of different analytes, using embossed omniphobic RF paper as a substrate, and pins as electrodes. Figure 5 shows that different wells can be used to perform independent analyses—cyclic voltammetry for the analysis of solutions of FcCO_2H and hydroquinone, respectively.

Approaches to multiplexing: Thread-based arrays of pin electrodes

[0091] Linear arrays of electrodes (carbon-coated stainless steel pins as working electrodes, and stainless steel pins as either counter or quasi-reference electrodes) can form interfaces with the liquid wicking along the same thread. The electrochemical cells within the thread-based arrays can be either linked or independent, such that each cell in a multiplex device can be used to perform, in rapid succession or simultaneously, independent measurements for one or several solutions of analyte along the same thread. Figure 6A and 6B show a device comprising multiple alternating stainless steel pins and carbon-coated stainless steel pins and a single thread that forms three helical turns around each pin; the device can be used for multiple measurements (in rapid succession) of the same analyte on thread.

[0092] Figure 6C shows chronoamperograms for the same solution of analyte, recorded, in succession, using each of the seven WEs positioned along a single thread. In each measurement, the two adjacent stainless steel pins served as CE and RE, such as each two successive cells share one stainless steel pin that serves as a counter electrode in the former and as reference electrode in the latter.

[0093] By introducing hydrophobic barriers along the thread, independent electrochemical cells capable of performing different analyses along the same thread can be formed. Figure 7A and 7B show a device having three distinct electrochemical cells formed from a single thread; arrows indicate the presence of a small amount of polymer (cyanoacrylate) that serves as a boundary between consecutive cells. The device can be used for measurement of three different analytes (in rapid succession or simultaneously). Figure 7C shows square-wave voltammograms recorded with each of the three electrochemical cells along a single thread, for three solutions with different concentrations of analyte: $10\ \mu\text{M}$ FcCO_2H in PBS, pH 7.6; buffer only, PBS, pH 7.6; and $100\ \mu\text{M}$ FcCO_2H in PBS, pH 7.6. There is no observable interference between neighboring cells.

[0094] Unless otherwise defined, used or characterized herein, terms that are used herein (including technical and scientific terms) are to be interpreted as having a meaning that is consistent with their accepted meaning in the context of the relevant art and are not to be interpreted in an idealized or overly formal sense unless expressly so defined herein. For example, if a particular composition is referenced, the composition may be substantially, though not perfectly pure, as practical and imperfect realities may apply; e.g., the potential presence of at least trace impurities (e.g., at less than 1 or 2%) can be understood as being within the scope of the description; likewise, if a particular shape is referenced, the shape is intended to include imperfect variations from ideal shapes, e.g., due to manufacturing tolerances. Percentages or concentrations expressed herein can represent either by weight or by volume.

[0095] Although the terms, first, second, third, etc., may be used herein to describe various elements, these elements are not to be limited by these terms. These terms are simply used to distinguish one element from another. Thus, a first element, discussed below, could be termed a second element without departing from the teachings of the exemplary embodiments. Spatially relative terms, such as “above,” “below,” “left,” “right,” “in front,” “behind,” and the like, may be used herein for ease of description to describe the relationship of one element to another element, as illustrated in the figures. It will be understood that the spatially relative terms, as well as the illustrated configurations, are intended to encompass different orientations of the apparatus in use or operation in addition to the orientations described herein and depicted in the figures. For example, if the apparatus in the figures is turned over, elements described as “below” or “beneath” other elements or features would then be oriented “above” the other elements or features. Thus, the exemplary term, “above,” may encompass both an orientation of above and below. The apparatus may be otherwise oriented (e.g., rotated 90 degrees or at other orientations) and the spatially relative descriptors used herein interpreted accordingly. Further still, in this disclosure, when an element is referred to as being “on,” “connected to,” “coupled to,” “in contact with,” etc., another element, it may be directly on, connected to, coupled to, or in contact with the other element or intervening elements may be present unless otherwise specified.

[0096] The terminology used herein is for the purpose of describing particular embodiments and is not intended to be limiting of exemplary embodiments. As used herein,

singular forms, such as “a” and “an,” are intended to include the plural forms as well, unless the context indicates otherwise.

[0097] It will be appreciated that while a particular sequence of steps has been shown and described for purposes of explanation, the sequence may be varied in certain respects, or the steps may be combined, while still obtaining the desired configuration. Additionally, modifications to the disclosed embodiment and the invention as claimed are possible and within the scope of this disclosed invention.

CLAIMS

1. An electroanalytical device, comprising:

a pin set comprising at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a tip; and

a paper substrate, wherein the substrate is shaped to provide at least one recess capable of holding a liquid,

wherein the shafts of two conductive pins traverse the paper substrate to anchor the heads of the two conductive pins on the recess surface.

2. The device of claim 1, wherein substrate is hydrophobic or omniphobic.
3. The device of claim 2, wherein the substrate comprises a surface treated to be hydrophobic or omniphobic.
4. The device of any of claims 1-3, wherein one or both of the conductive pins for use as working and counter electrodes comprise stainless steel pins.
5. The device of any of claims 1-3, wherein one or both of the conductive pins for use as working and counter electrodes comprise a conductive coating.
6. The device of claim 5, wherein the coating is selected from the group consisting of conductive carbon.
7. The device of claim 6, wherein the carbon coating is selected from the group consisting of graphite and carbon nanotubes and mixtures thereof
8. The device of any of claims 1-7, wherein the pin set further comprises a third conductive pin for use as a reference electrode, wherein the third pin is comprised of a head, a shaft and a tip and wherein the shaft of the third pin traverses the paper substrate to anchor the head of the third conductive pin on the well surface.
9. The device of claim 8, wherein the working electrode conductive pin comprises a conductive coating.

10. The device of any of claims 3, wherein the hydrophobic or omniphobic paper substrate is chemically functionalized with hydrophobic or omniphobic functional moieties.
11. The device of any of claims 1-10, wherein the recess is embossed into the paper substrate.
12. The device of any of claims 1-10, wherein the recess is a well or a channel.
13. The device of any of claims 1-10, wherein the paper substrate comprises a plurality of recesses.
14. The device of claim 13, wherein each of the recesses contains a pin set.
15. The device of claim 13, wherein the device is a 96 well device.
16. The device any of claims 1-15, further comprising one or more reagents located in the recess, wherein the reagents are selected to interact with an analyte of interest.
17. A method of making an electroanalytical device, comprising:

providing at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a piercing tip; and

inserting each of the pins into a recess in a hydrophobic or omniphobic paper substrate, and locating the pin head in the recess of the paper substrate.
18. A method of electroanalysis, comprising:

providing an electroanalytical device according to any one of claims 1-16; and

introducing a liquid into the recess of the device; and

reading out an electrical signal, said signal indicating a property or state of the liquid analyte.
19. An electroanalytical device, comprising:

a pin set comprising at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a tip;

a thread, serially wound around the shafts of each of the two conductive pins; and
a base into which the tip of each of the pins is secured.

20. The device of claim 19, wherein one or both of the conductive pins for use as working and counter electrodes comprise stainless steel pins.
21. The device of claim 19, wherein one or both of the conductive pins for use as working and counter electrodes comprises a conductive coating.
22. The device of claim 21, wherein the coating is selected from the group consisting of conductive carbon.
23. The device of claim 22, wherein the carbon coating is selected from the group consisting of graphite and carbon nanotubes and mixtures thereof.
24. The device of claim 19-23, wherein the pin set further comprises a third conductive pin for use as a reference electrode, wherein the third pin is comprised of a head, a shaft and a tip and wherein the shaft of the third pin traverses the paper substrate to anchor the head of the third conductive pin on the well surface.
25. The device of claim 24, wherein the working electrode conductive pin comprises a conductive coating.
26. The device of any of claims 19-25, wherein the device includes a plurality of conductive pin sets, and the thread is wound in series around each of the pin sets.
27. The device of any of claims 26, further comprising a barrier located on the thread between pin sets.
28. The device of any of claims 19-27, wherein the device includes a plurality of conductive pin sets, and a plurality of thread and each thread is wound around one pin set.
29. A method of making an electroanalytical device, comprising:

providing at least two conductive pins for use as working and counter electrodes, wherein the first and second pins are comprised of a head, a shaft and a piercing tip;

inserting each of the pins into a base; and

serially winding a thread around the shafts of each of the two conductive pins.

30. A method of electroanalysis, comprising:

providing an electroanalytical device according to any one of claims 19-28; and

introducing a liquid the thread of the device; and reading out an electrical signal, said signal indicating a property or state of the liquid analyte.

FIG. 1A

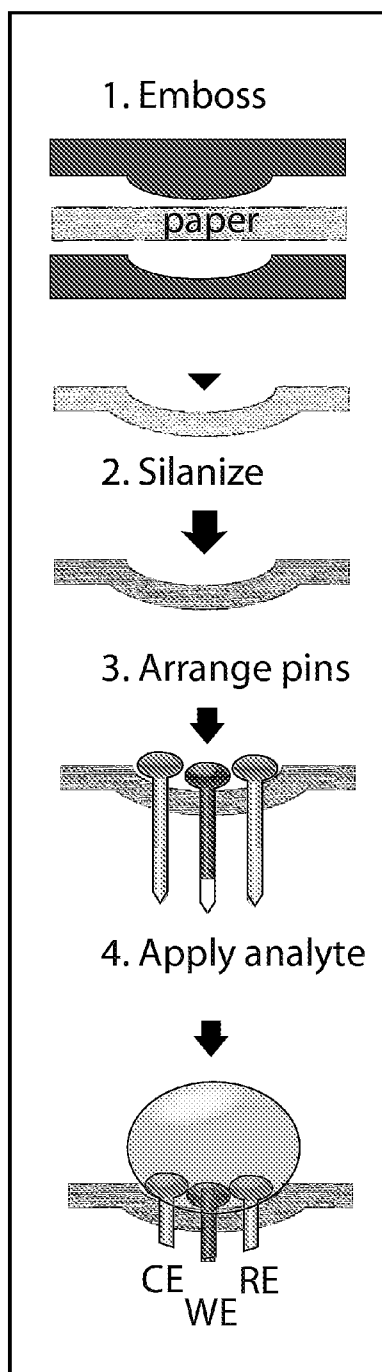


FIG. 1B

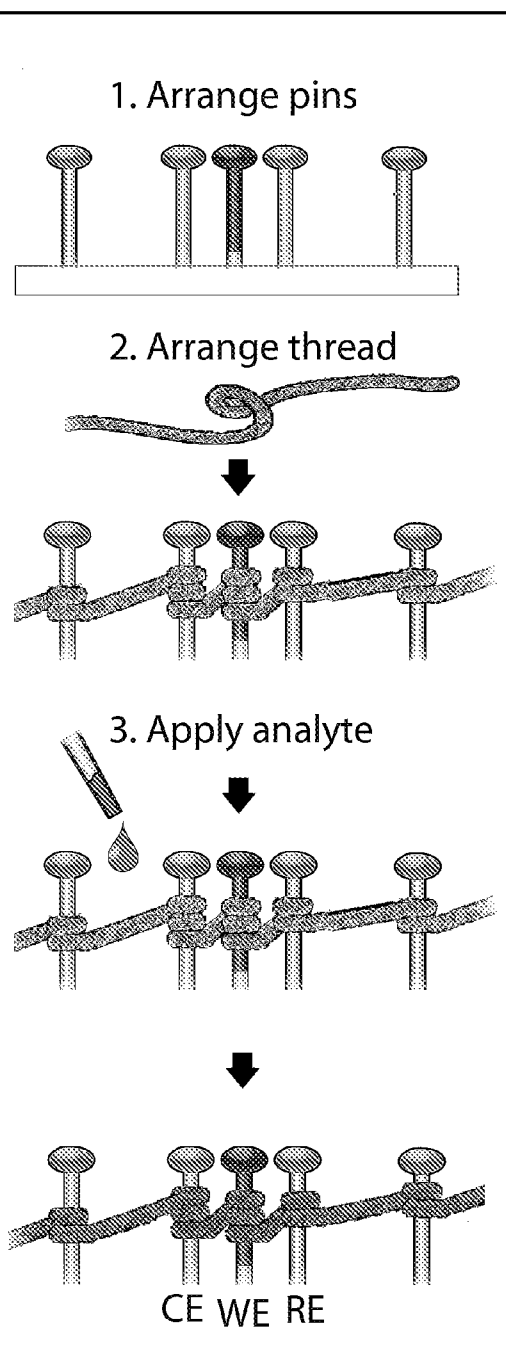


FIG. 2A

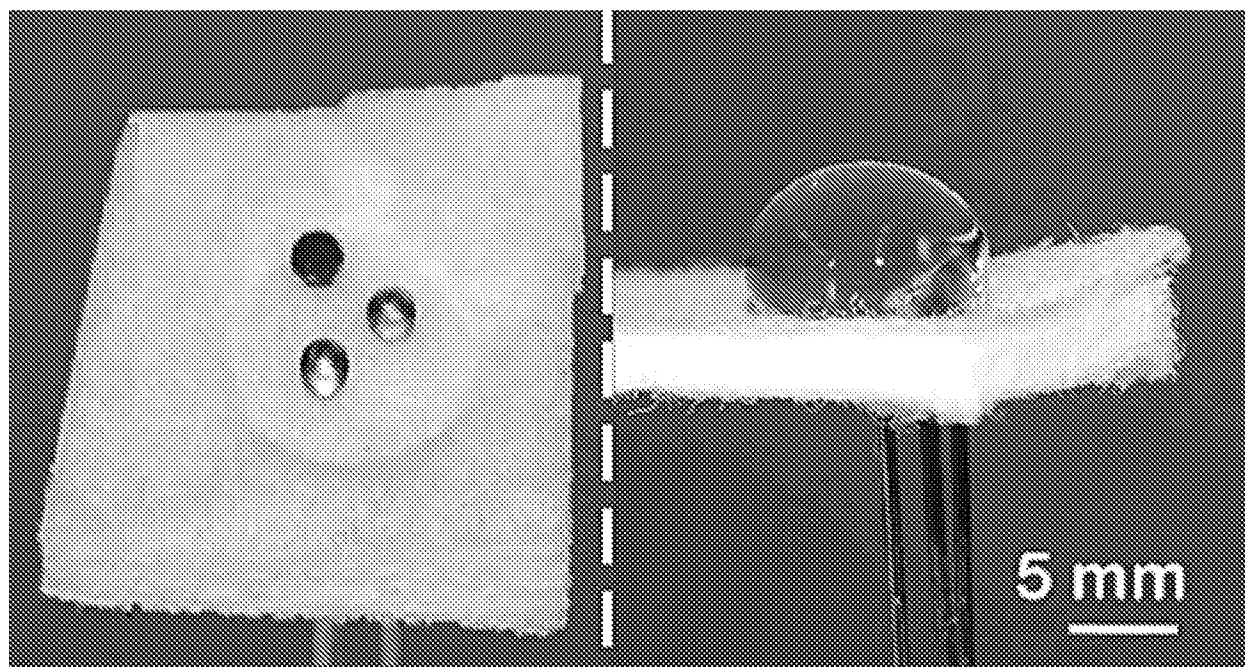


FIG. 2B

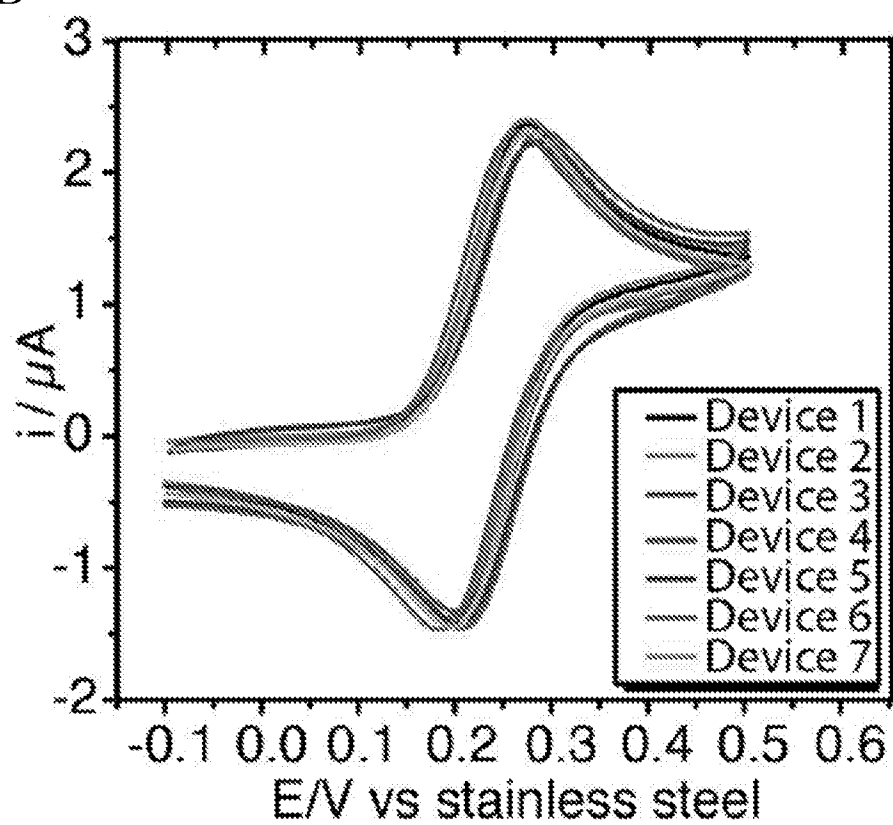


FIG. 2C

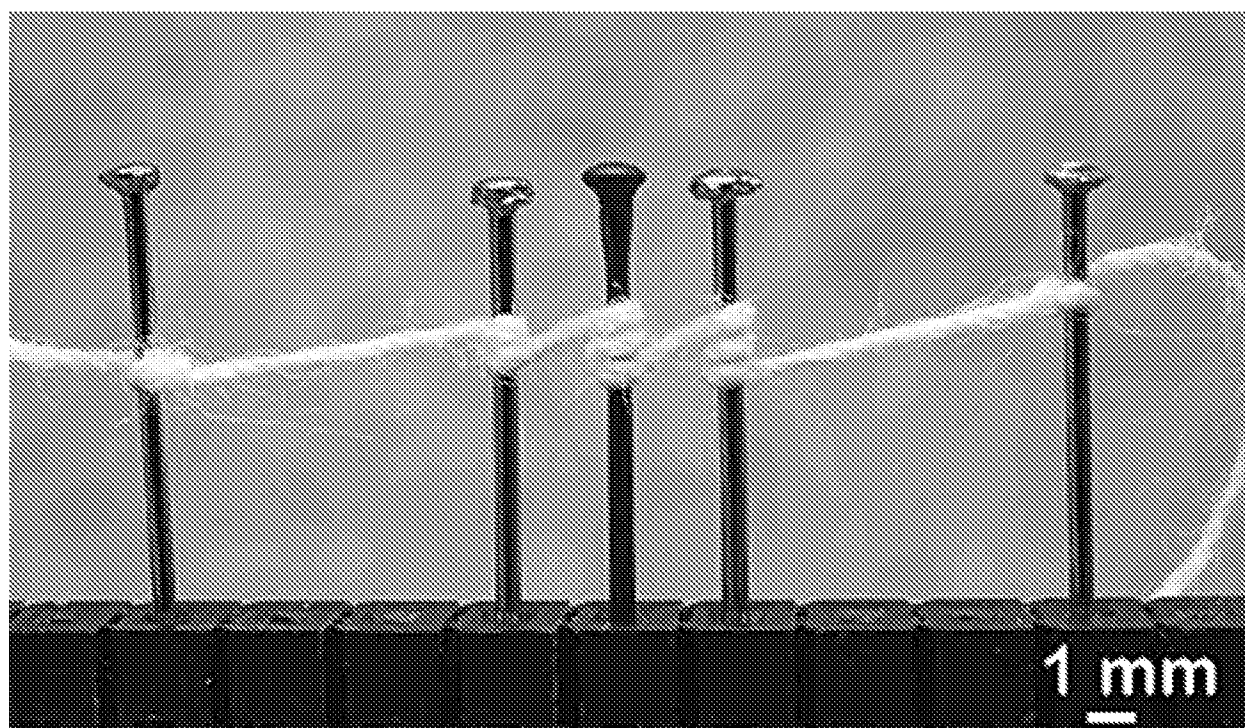


FIG. 2D

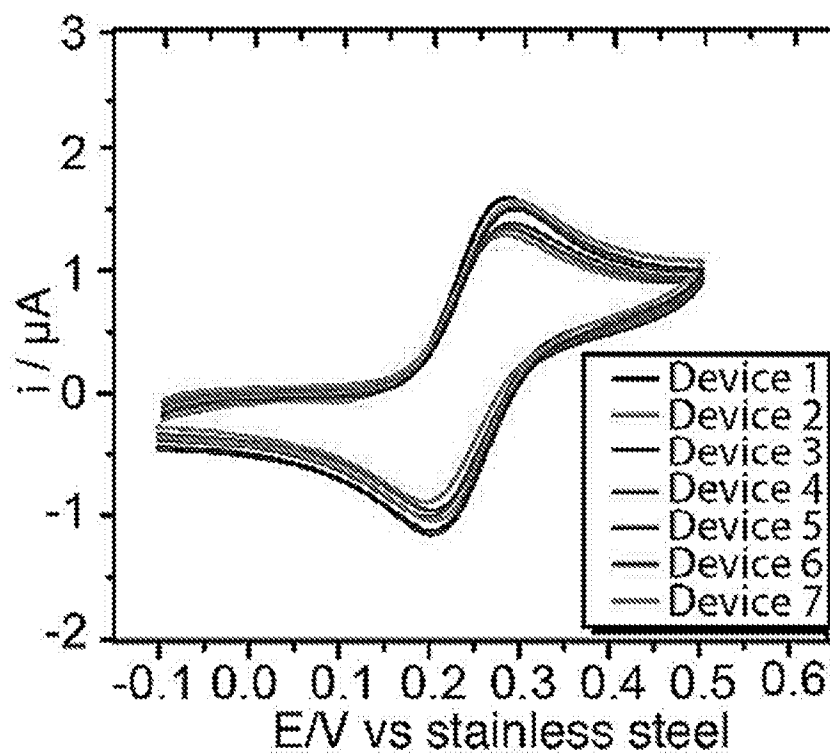


FIG. 3A

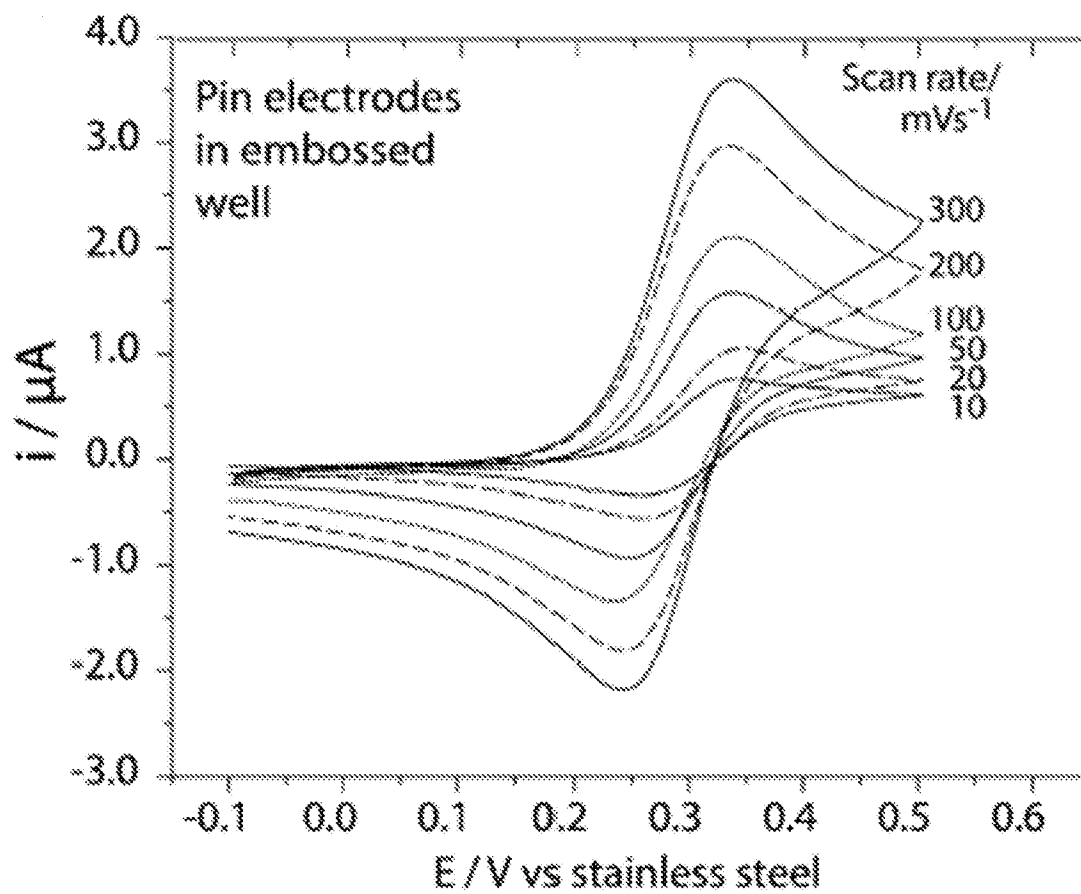


FIG. 3B

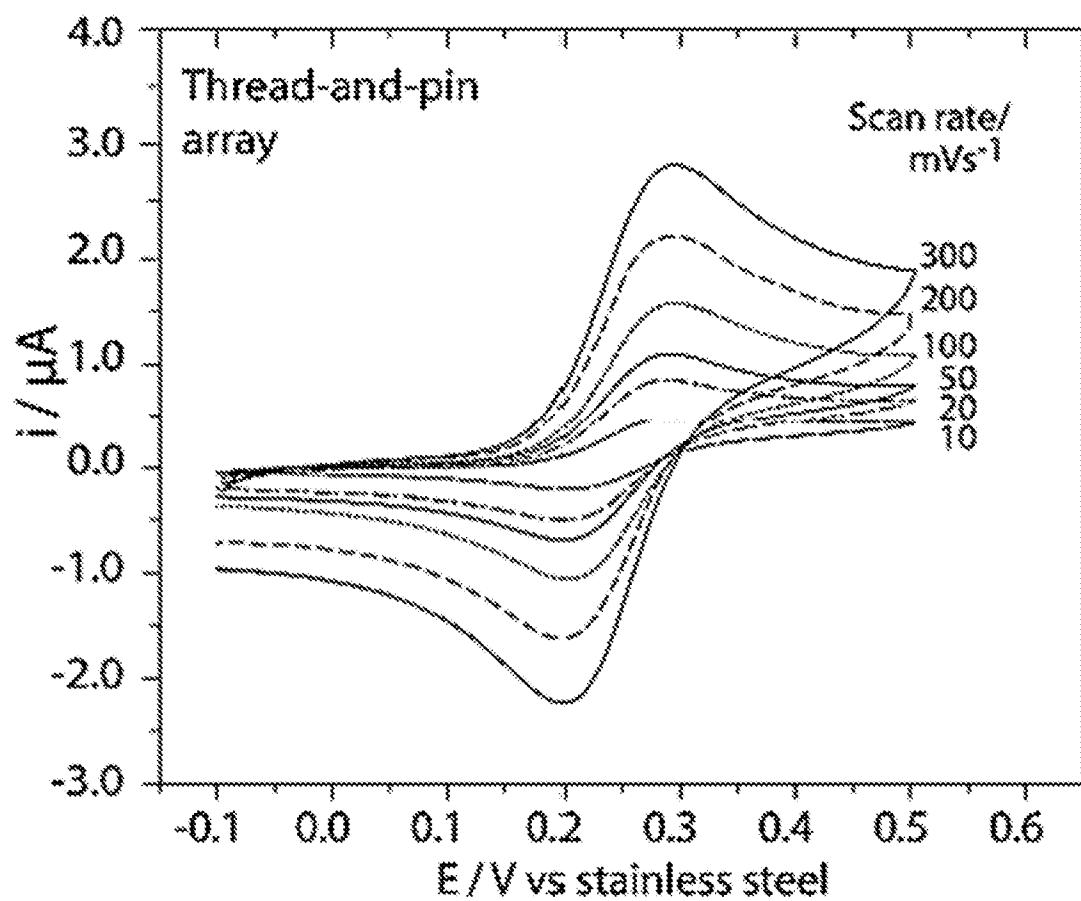


FIG. 3C

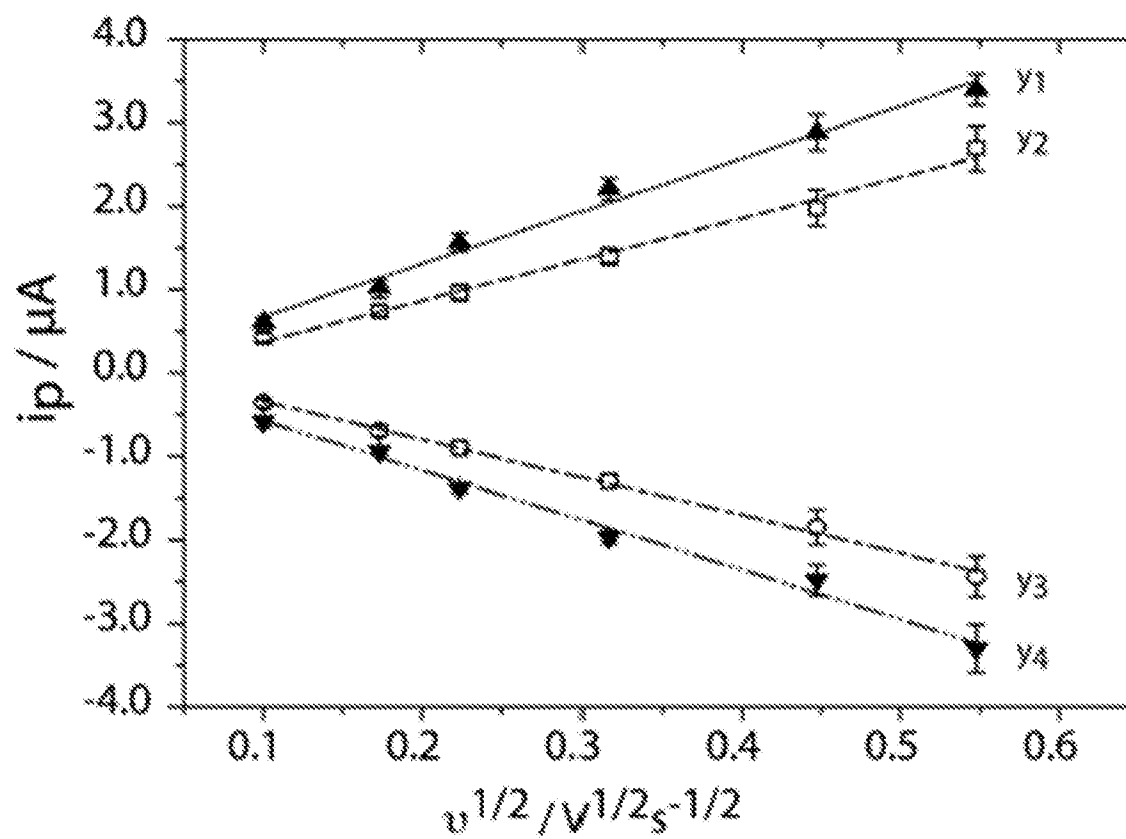


FIG. 4A

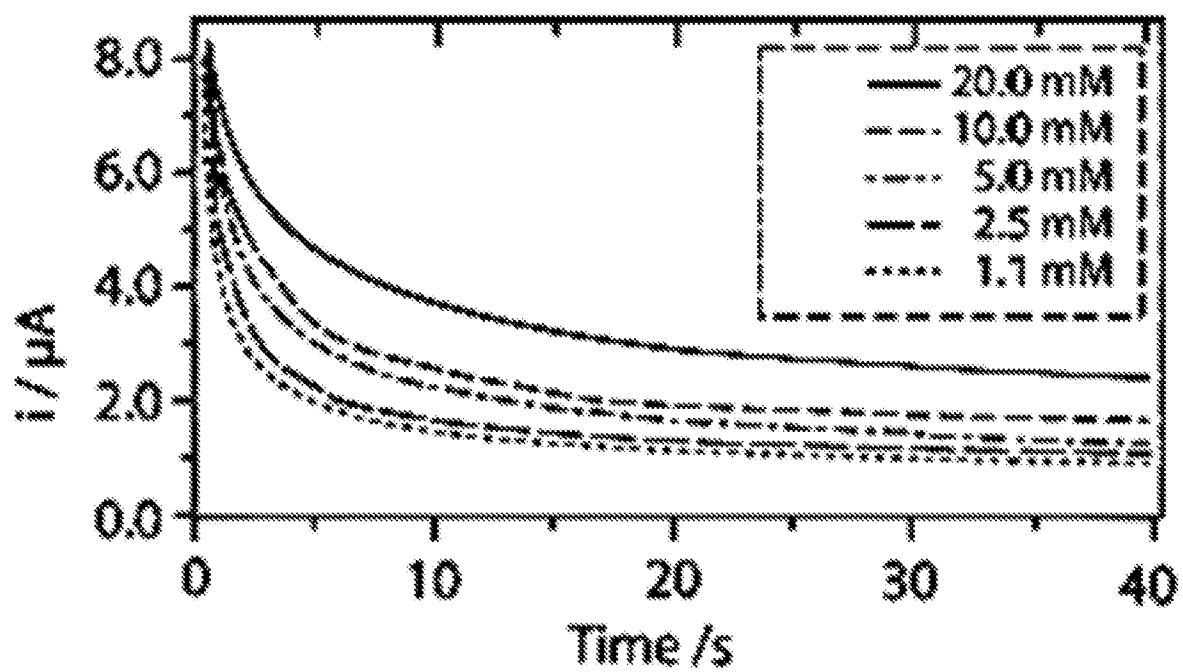


FIG. 4B

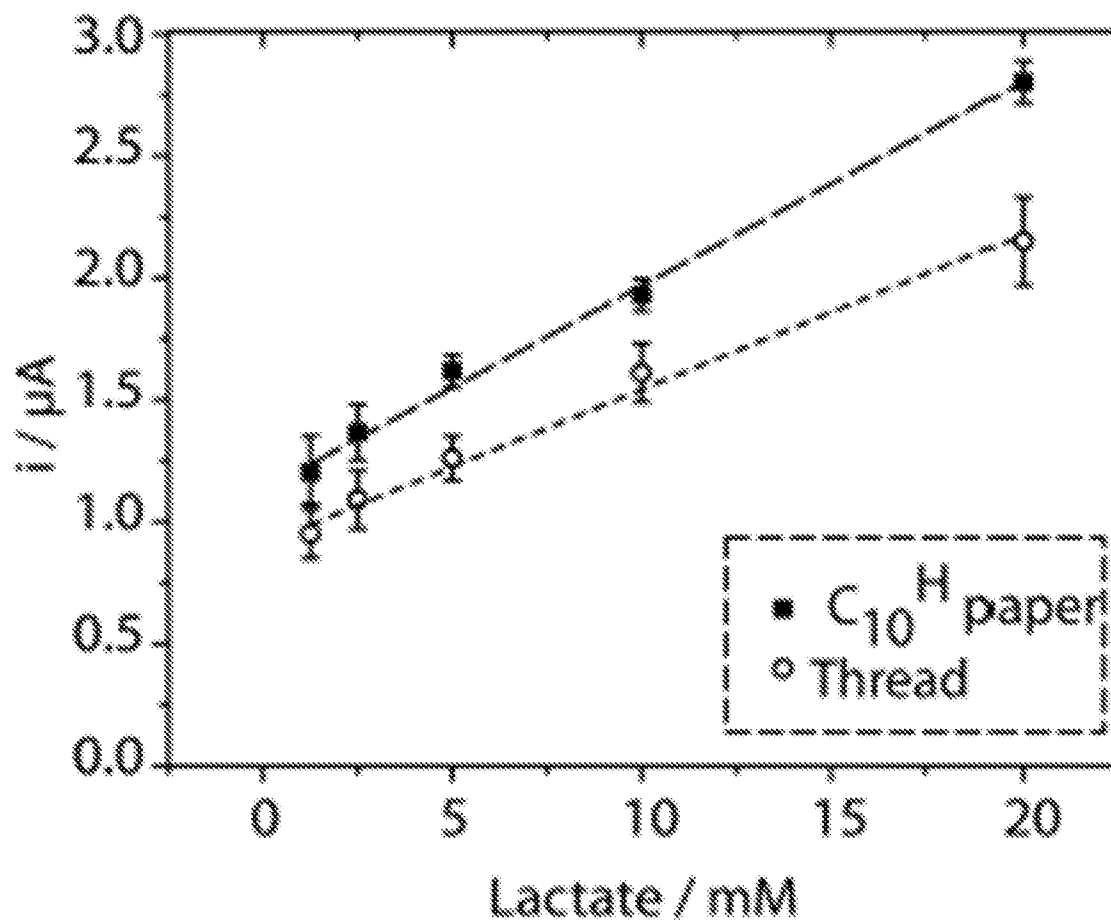


FIG. 5A

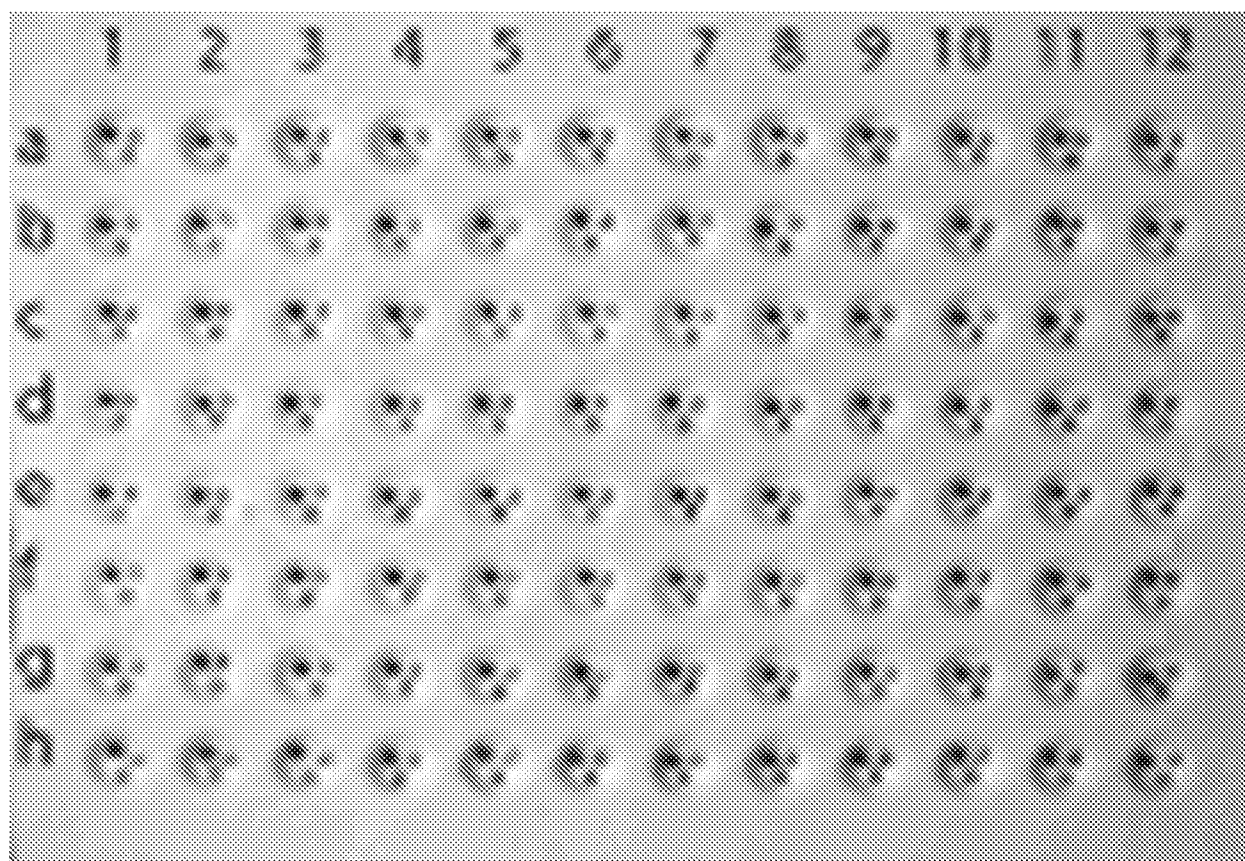


FIG. 5B

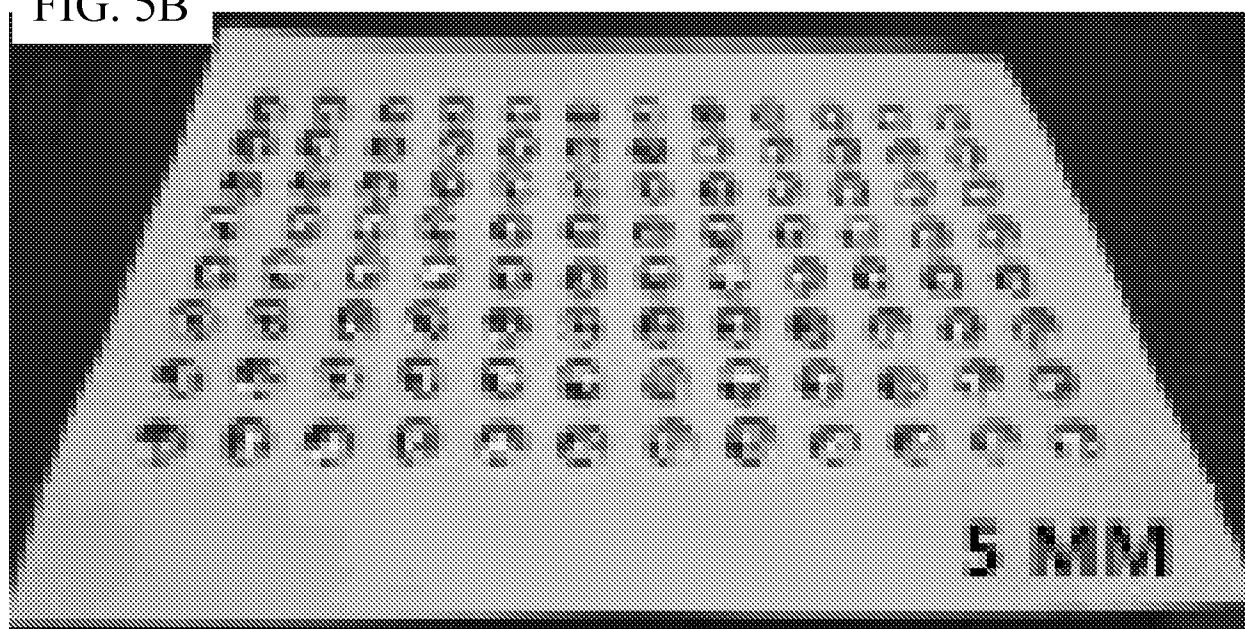


FIG. 5C

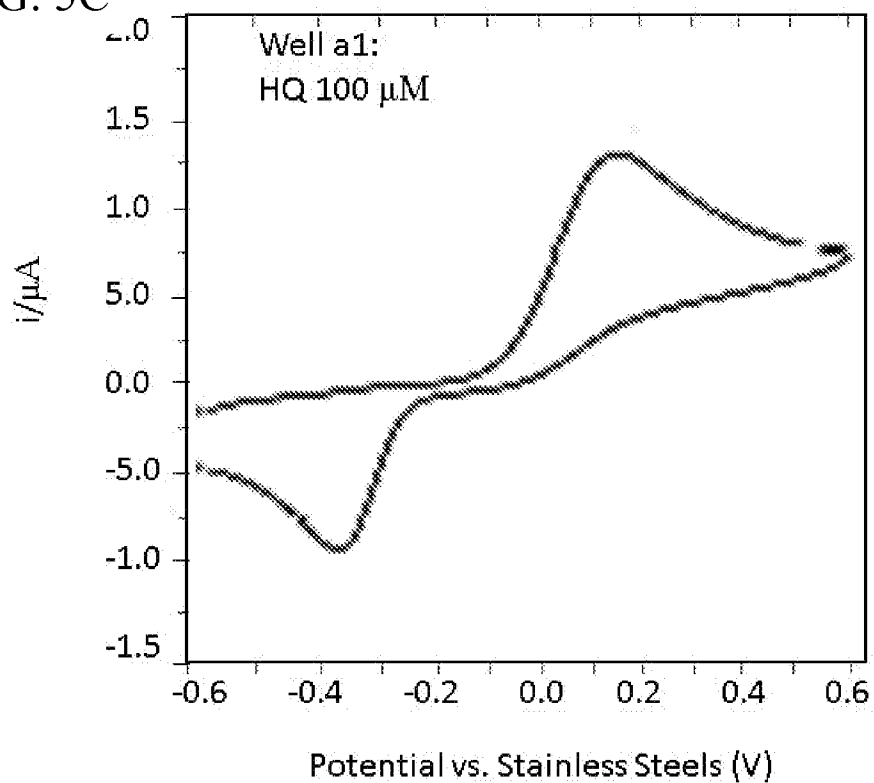


FIG. 5D

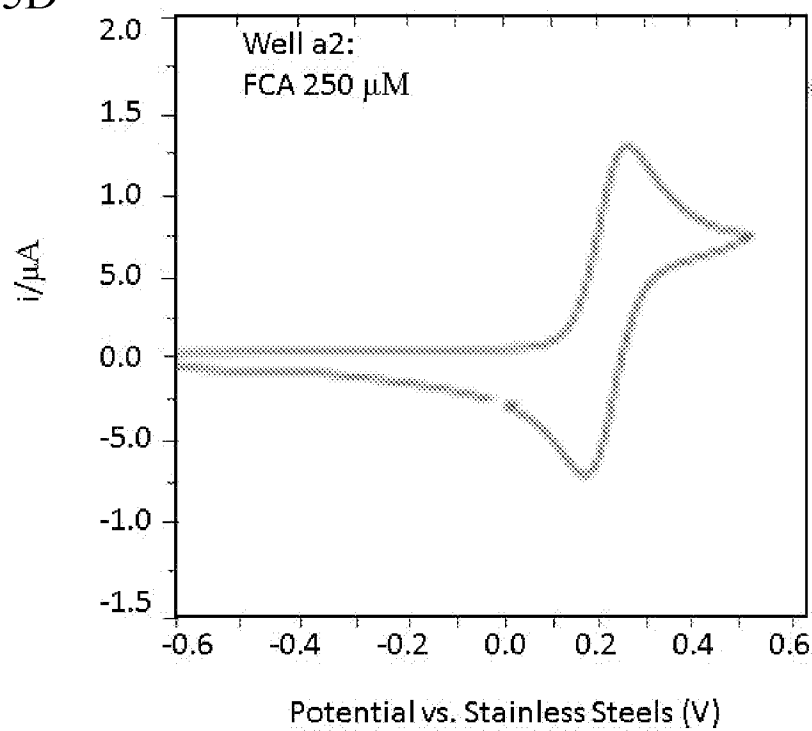


FIG. 6A

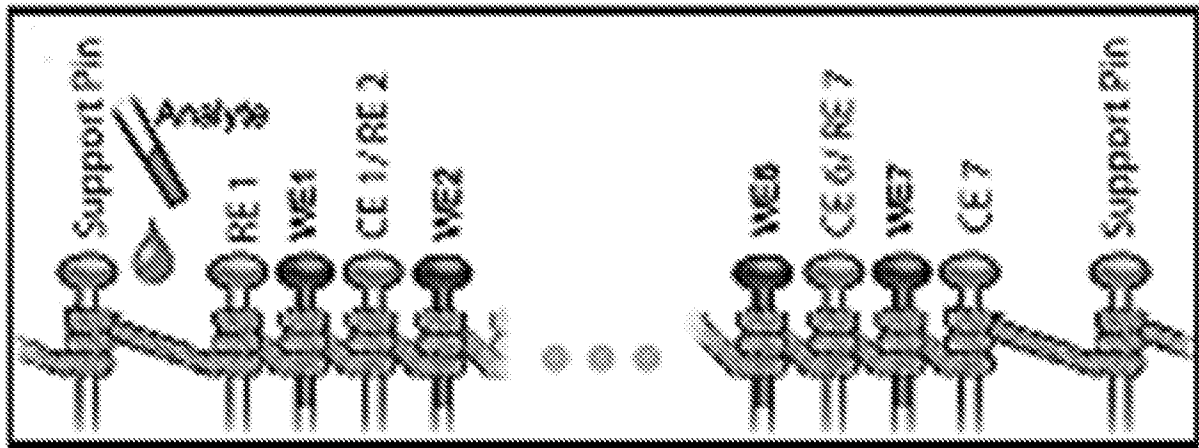


FIG. 6B

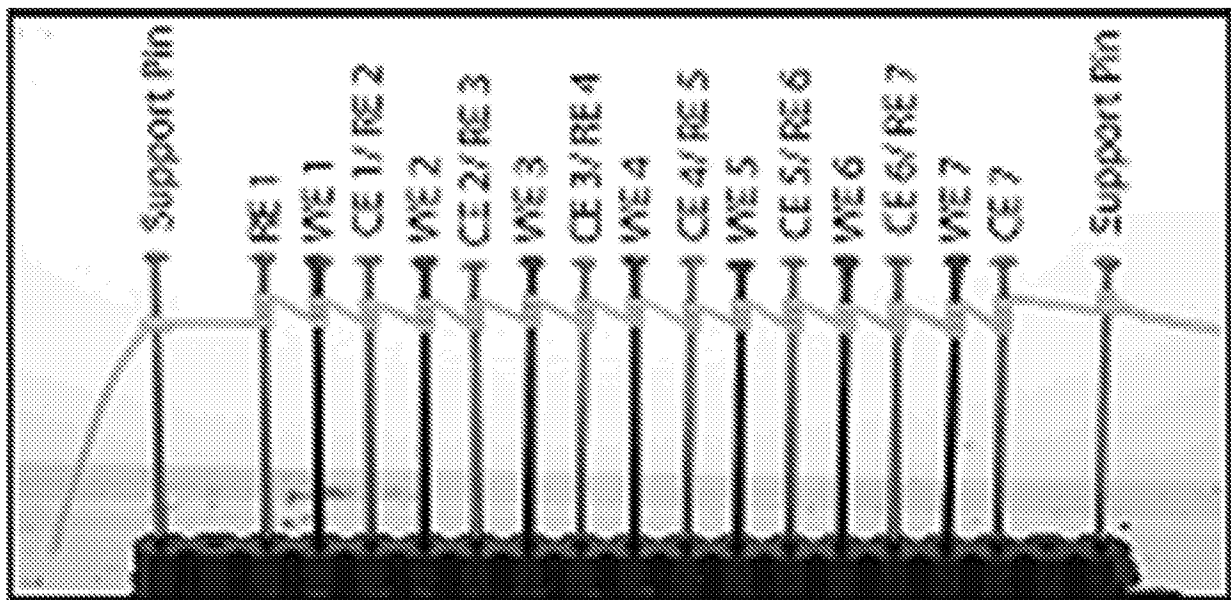


FIG. 6C

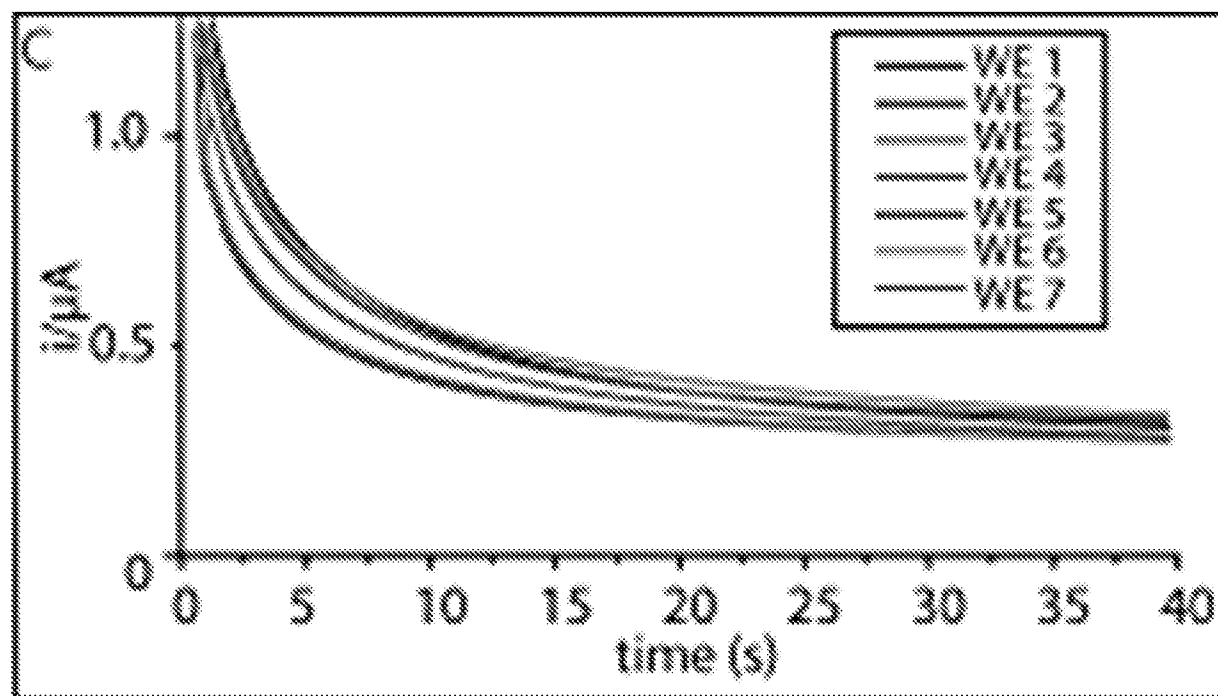


FIG. 7A

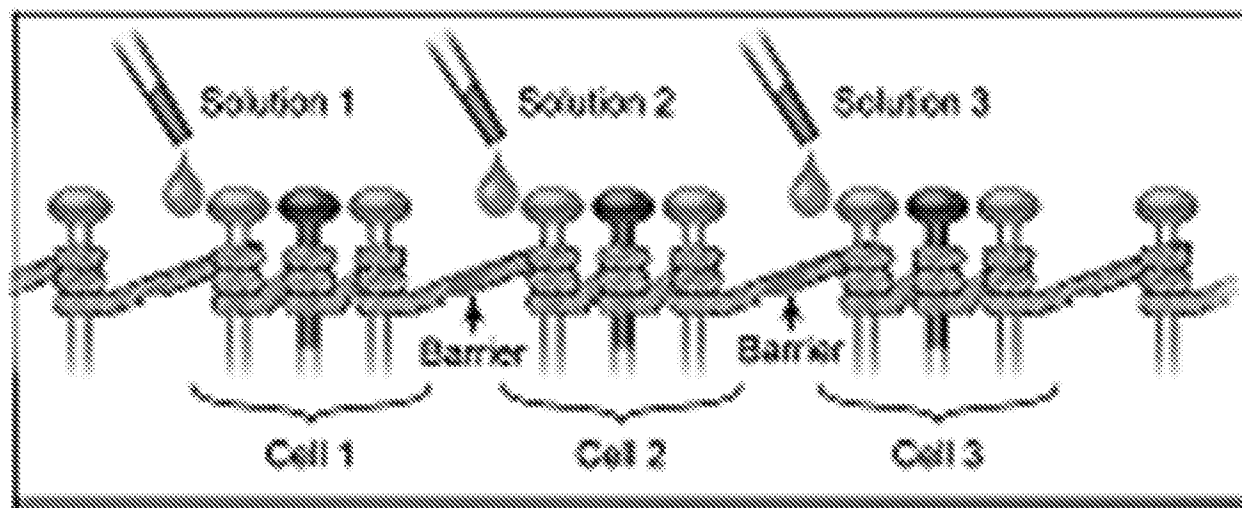


FIG. 7B

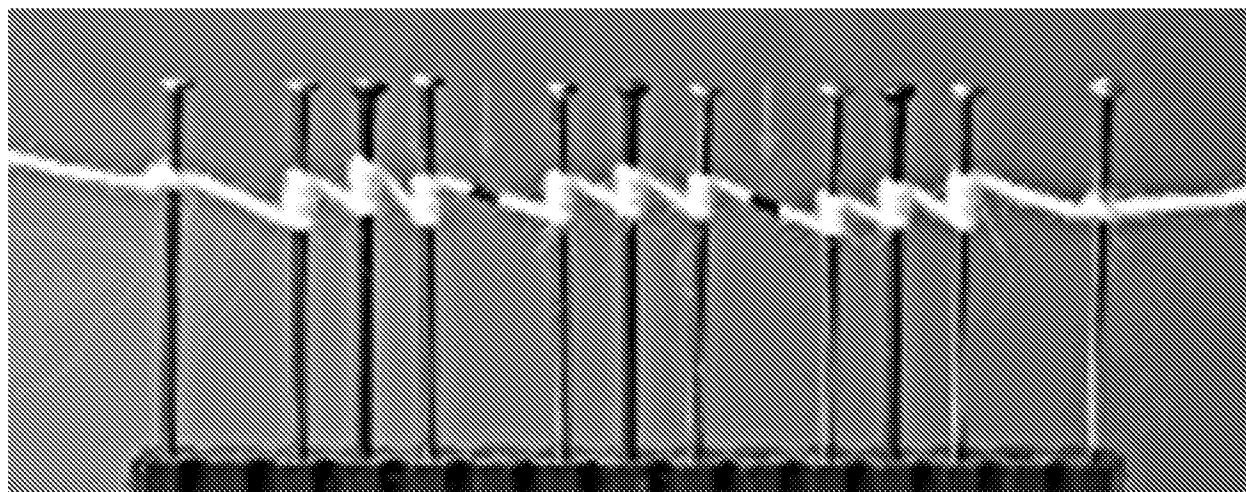


FIG. 7C

