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New Method in Surface Treatment of Nanopipette for Interface between Two Immiscible Electrolyte Solutions (ITIES) Experiment

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Interface between two immiscible electrolyte solutions (ITIES) is a powerful platform for chemical sensing and studying electron/ion transfer reactions and is typically formed between the interface of two immiscible solutions such as an oil phase and an aqueous phase. Micro/nano ITIES interface are generally formed at the tip of a borosilicate/quartz pipette, inner surface of which can be rendered hydrophobic to be filled with an organic solvent by a method called silanization. Nano/micrometer-sized electrodes are typically silanized by vapor silanization methods in which silanizing agent in vapor phase is exposed to nanopipettes. Micrometer-sized pipettes have been also silanized by directly filling liquid silanization agent, one type of liquid silanization methods, but this method has not been used at the nanoscale. Liquid silanization method allows to selectively silanize a single channel in a dual-channel pipette platform. Here, we developed the liquid silanization method for nanoscale ITIES and demonstrated that a stable cyclic voltammogram for tetrabutylammonium ion transfer across water/dichloroethane interface can be accomplished. We also presented challenges for liquid silanization at the nanoscale and strategies to overcome them. The liquid silanization methods presented here lay the foundation for future development of dual channel multi-functional probe where one channel is nanoITIES.
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A nano/microITIES electrode surface is a liquid/liquid junction of two immiscible electrolyte solutions formed at the tip end of a nano/micropipette. Generally, the interface is formed between an organic phase and an aqueous phase at the pipette tip. Pipettes have been filled with either an organic or an aqueous phase. For the interface to remain stable, it must be controlled to prevent the solution interface from receding into or protruding from the pipette during experiments.[1] Pipettes are typically made with quartz or glass capillaries whose surface is hydrophilic with –OH groups. To obtain a stable interface at the tip of the pipette, surface modification of the pipettes is necessary by a process called silanization. For silanization, silanization agents such as N,N-dimethyltrimethylsilylamine or chlorotrimethylsilane (TMS-Cl) reacts with hydrophilic –OH groups on the surface of the glass and replaces –OH groups with hydrophobic silyl moieties.[2–5] Silanization can be classified into two types based on the physical state of the silanization agent, i.e., vapor and liquid silanizations, respectively.

In 1977, Coles and Tsacopoulos developed a chemical vapor silanization method for micropipettes, where the vapor was generated by passing N_2 through a silanization agent, i.e., dichlorodimethylsilane.[6] Later on in 1980, Tsein and Rink developed a modified method. They produced silane vapor by using high temperature and trapped the silane vapor inside an inverted beaker where pulled micrometer-sized pipettes were kept.[7] In 2010, Mirkin and Amemiya groups reported vapor phase silanization where nanopipettes were kept 1–2 cm above the surface of silane solution (e.g., TMS-Cl) while passing argon through the back of the pipette.[8] Amemiya group further developed a different vapor phase silanization method for nanometer-sized pipettes, which has been used by us and others.[9–13] In this method developed by the Amemiya group, a plastic desiccator is used as a reactor where the pulled pipettes are placed, and a vacuum is created in the desiccator to introduce the vapors of the silane thus the pipettes are exposed to the silane vapor for surface modification.[10]

Instead of silane vapor, liquid silane has also been used for micropipettes generally called liquid silanization methods. Pipettes can be silanized by immersion in a silane solution rendering both the inner and outer surfaces of the pipette hydrophobic.[14] This method has been slightly modified to silanize only the outside of the pipette by passing a neutral gas like argon or nitrogen through the pipette while it is immersed in the silane solution, preventing the silanization of the internal pipette surface.[2–5,15,16] This can be useful if the pipettes will be filled with an aqueous solution, as demonstrated by recent work where nanopipettes were immersed in the organic solution and aqueous solution was filled inside the pipette.[15,16] In addition, the inner surfaces of micropipettes with radii ranging from 3–25 μm were rendered hydrophobic by inserting TMS-Cl from the back of the pipette using a syringe and drying it overnight.[4] This method is useful for the ITIES configuration with the organic phase contained inside the pipette and the aqueous phase outside. However, silanization using this method has yet to be reported for nanometer-sized pipettes.

Apart from single-channel nanopipettes, scientists have been developing dual functional nanopipettes which are produced by pulling theta capillaries using a laser puller. Since ITIES is a very powerful analytical platform for chemical sensing, studying different electron/ion transfer reactions, detecting neurotransmitters during exocytosis, etc., one chamber of the dual channel pipette can be modified to serve as an ITIES electrode while the second channel can be developed for completely different use. The second channel can either be modified to a solid electrode by filling platinum or carbon which can be used to detect redox active analyte molecules or it can serve as delivery channel to deliver chemicals in a spatially and volumetrically controlled fashion. The vapor silanization method currently used for nanopipettes silanizes both channels of the pipette which may affect the second channel (non-ITIES). Thus, silanization method which selectively silanize only one channel of the dual channel pipette has to be developed. While liquid silanization has been proven successful for modifying the inner surface of microITIES, this method has yet to be reported for nanometer-sized pipettes. In this work, we developed liquid silanization method for nanoITIES, where we also presented technical challenges and strategies to overcome these.

Experimental

Materials and reagents.—Sodium chloride (NaCl) was from EMD Chemicals (Gibbstown, NJ). Potassium chloride (KCl) was from VWR International (Radnor, PA). Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) was from Amresco (Solon, OH). Magnesium sulfate ($MgSO_4$) and 4-(2-hydroxyethyl)-1-pipera-zineethanesulfonic acid (HEPES) were from Fischer Scientific (Pittsburgh, PA). Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), tetradodecylammonium chloride (TDDACl), tetrabutylammonium chloride (TBACl), 1,2-dichloroethane (DCE), potassium tetrakis

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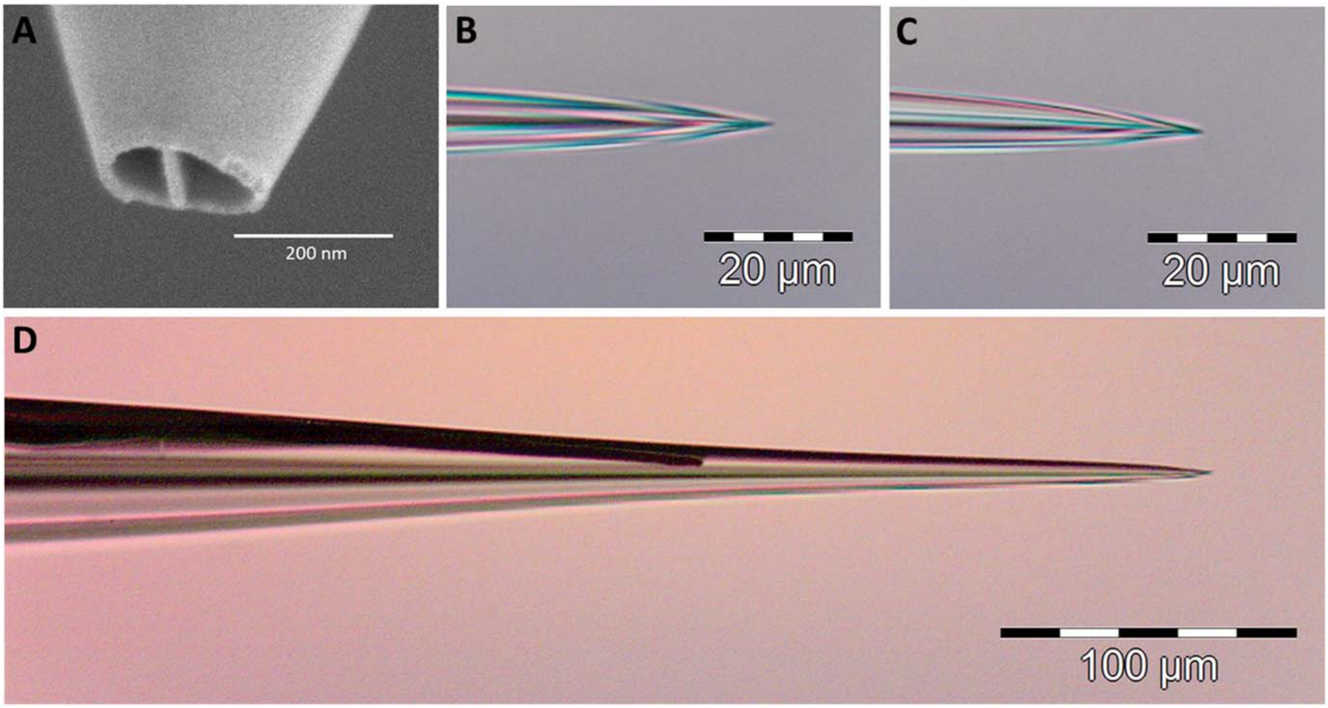

Figure 1. SEM image of the dual-channel nanopipette (A). Microscopic images of the dual channel pipette after silanization and complete drying of ITIES channel (top channel) (B) and after filling DCE filling solution in the silanized channel (C) and after positioning etched Pt wire at 100–200 μm away from the tip end (D).

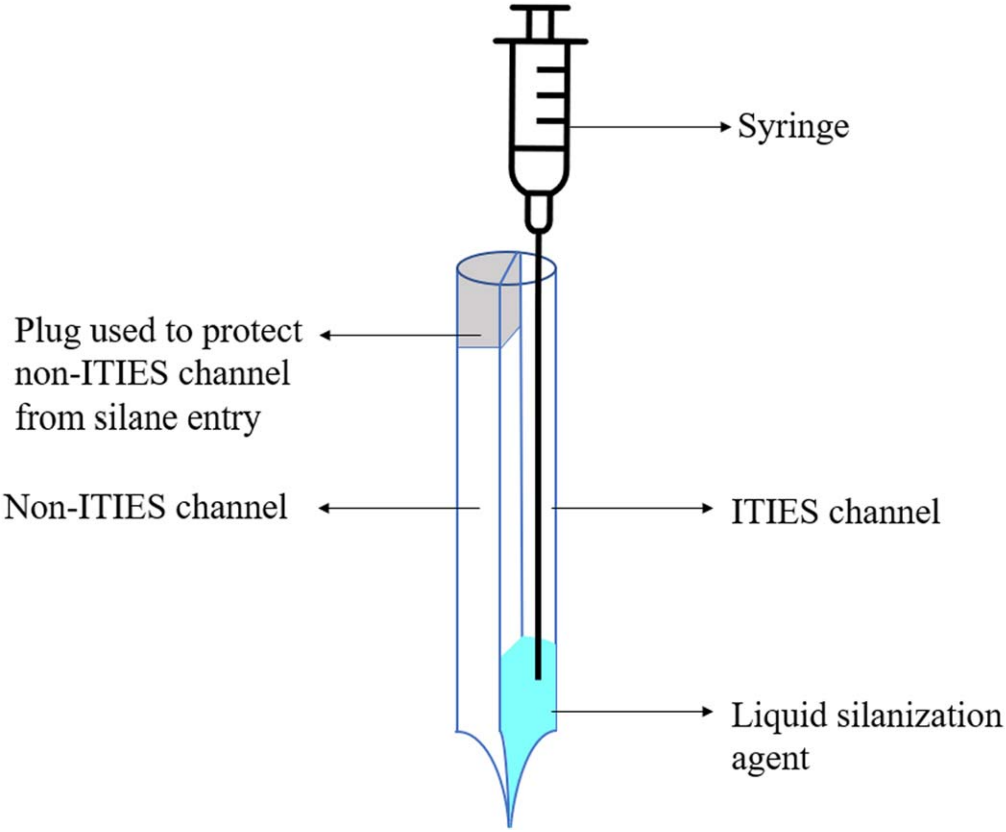

Figure 2. Schematic diagram of the process of silane filling into ITIES channel of dual-channel nanopipette using a syringe. The non-ITIES channel is blocked with a plug to prevent entry of silane.

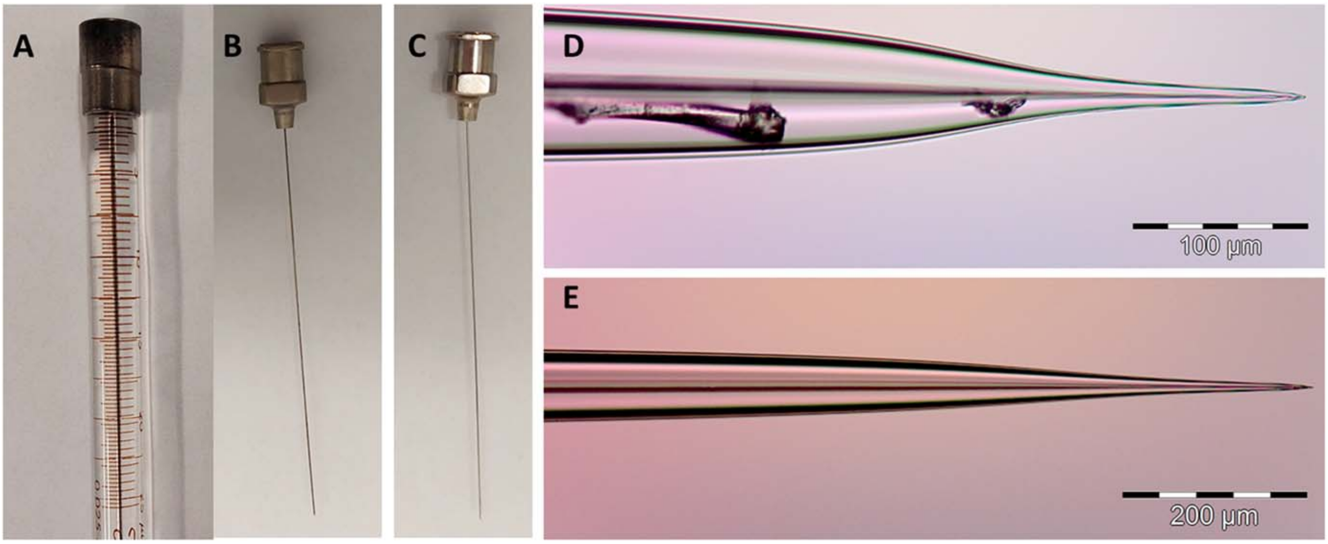

Figure 3. Corrosion of metal parts of the syringe by silane (TMS-Cl) and strategies to prevent that. The metal part of the luer lock (A) and the needle (B) of TMS-Cl dispensing syringe stored in open air under atmospheric conditions (20 °C–25 °C) appeared to be corroded whereas the needle stored in refrigerator (4 °C) and immersed in acetone (C) had a shiny appearance similar to brand new ones. Silanization of ITIES channel using syringe parts stored in atmospheric condition (appearance of corrosion) resulted in the presence of particles in the ITIES channel (bottom) after silanization (D). In contrast, silanization of a pipette using the acetone protected needle (no obvious corrosion) produced a clean ITIES channel (E).

(pentafluorophenyl) borate (TFAB) were from Boulder Scientific Company (Mead, CO). All aqueous solutions were prepared from 18.3 MΩ cm deionized water. The artificial seawater (ASW) used was an aqueous solution containing 460 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 22 mM $MgCl_2$, 26 mM $MgSO_4$, and 10 mM HEPES (pH 7.8). The TFAB salt of TDDA (TDDATFAB) was prepared by metathesis.[11,17–19] A 25 μl luer lock syringe with a 31gauge metal needle of Hamilton was used for injecting Chlorotrimethylsilane (TMS-Cl) from Sigma Aldrich during liquid silanization and also injecting DCE filling solution for the nanoITIES electrode.

Instruments for the measurements.—All electrochemical measurements were performed using a CHI 920D potentiostat (CH Instruments, Austin TX). Electrochemical characterization of nanoITIES channel was carried out by obtaining cyclic voltammograms of tetrabutylammonium ion (TBA) transfer at the DCE/water nanoITIES. A scanning electron microscope (SEM) (FEI Helios 600i Dual Beam SEM/FIB, FEI Co., Hills-boro, OR) was also used to visually confirm the radii of the nanopipette orifices. Pipettes were coated with gold and palladium mixture of 6:4 composition using a sputter coater (Emitech K575, Emitech Ltd., Ashford, Kent) under 20 mA current for 45 s before SEM. Sputter coating increases the resolution of the images that can be obtained using SEM by limiting charging on the glass pipette surface to reduce the blurring of the electron image. SEM images were captured under a 2 kV electron beam with a beam current of 43 pA.

Fabrication of dual channel nanopipettes.—Dual-channel nanopipettes were prepared by laser pulling quartz theta capillaries (Sutter Instruments Co. Novato CA; 1.2 mm outer diameter, septum thickness of 0.15 mm, 7.5 cm length) using a P-2000 laser Puller (Sutter Instruments, Novato, CA). The following pulling parameters were used to fabricate the dual-channel nanopipette: heat = 750, filament = 4, velocity = 64, delay = 135 and pull = 79.

Assembly of ITIES electrodes after liquid silanization.—After liquid silanization of one channel of the dual-channel nanopipette, the channel was assembled to form an ITIES electrode. The ITIES channel of the nanopipette was backfilled with an organic solution of 5 mM TDDATFAB dissolved in DCE (Fig. 1C). The solution was forced to the tip of the nanopipette using gentle vibrations. An etched Pt wire was inserted into the DCE filled pipette and positioned 100–200 μm away from the tip end as reported in previous works (Fig. 1D).[11,19,20] The tip of the nanoITIES electrode was inserted into artificial sea water (ASW) for electrochemical characterization. ASW was used as the aqueous medium since it is the cellular medium for *Apylsia californica*, common biological model used in our lab and many other groups for neuronal studies.[21–28] Cyclic voltammogram was obtained for the transfer of TBA from ASW (aqueous phase) into the pipette (organic phase) and was used for the electrochemical characterization of the liquid-silanized nanopipette. A three electrode configuration was used, where the commercial Ag/AgCl/KCl(1 M) electrode acted as the outer reference electrode. Salt bridge was employed in connecting outer reference electrode to ASW and a tungsten wire was used as the outer counter electrode. The applied negative potentials yielded positive ion transfer from ASW to DCE, which is presented as positive current as commonly reported.[4,19,20,29]

Results and Discussion

Liquid silanization of nanopipette and challenges.—We first fabricated dual-channel nanopipette by laser pulling a theta pipette. We used SEM to check the pulled theta pipettes. Based on the SEM results, pulling parameters were adjusted to produce a dual-channel nanopipette with both channel open and similar in size. As shown by SEM results in Fig. 1A, the dual-channel nanopipette we produced had semicircular openings with the distance from the inner septum to the outer wall ranging from ∼50–150 nm measured based on multiple dual channel pipettes.

Then we tested liquid silanization in one channel of the dual-channel nanopipettes to find the best silanization condition, thus a stable ITIES can be formed, which is essential for the performance of the ITIES electrode. TMS-Cl was loaded in the syringe and the needle was inserted into the back of the pipette and the silane was pushed towards the tip end by the piston (Fig. 2). To prevent liquid silanization agent from entering the second channel through the back of the pipette during this process, we blocked the back side of the non-ITIES channel of dual-channel nanopipette with a plug made of 1:1 mixture of wall mounting putty and dental wax. After filling the silane, we first laid down the pipettes in horizontal configuration during which the silane evaporated quickly (within two minutes), since the area of exposure of the silane to the atmosphere was larger, this resulted in under-silanized pipettes. Later, we modified the

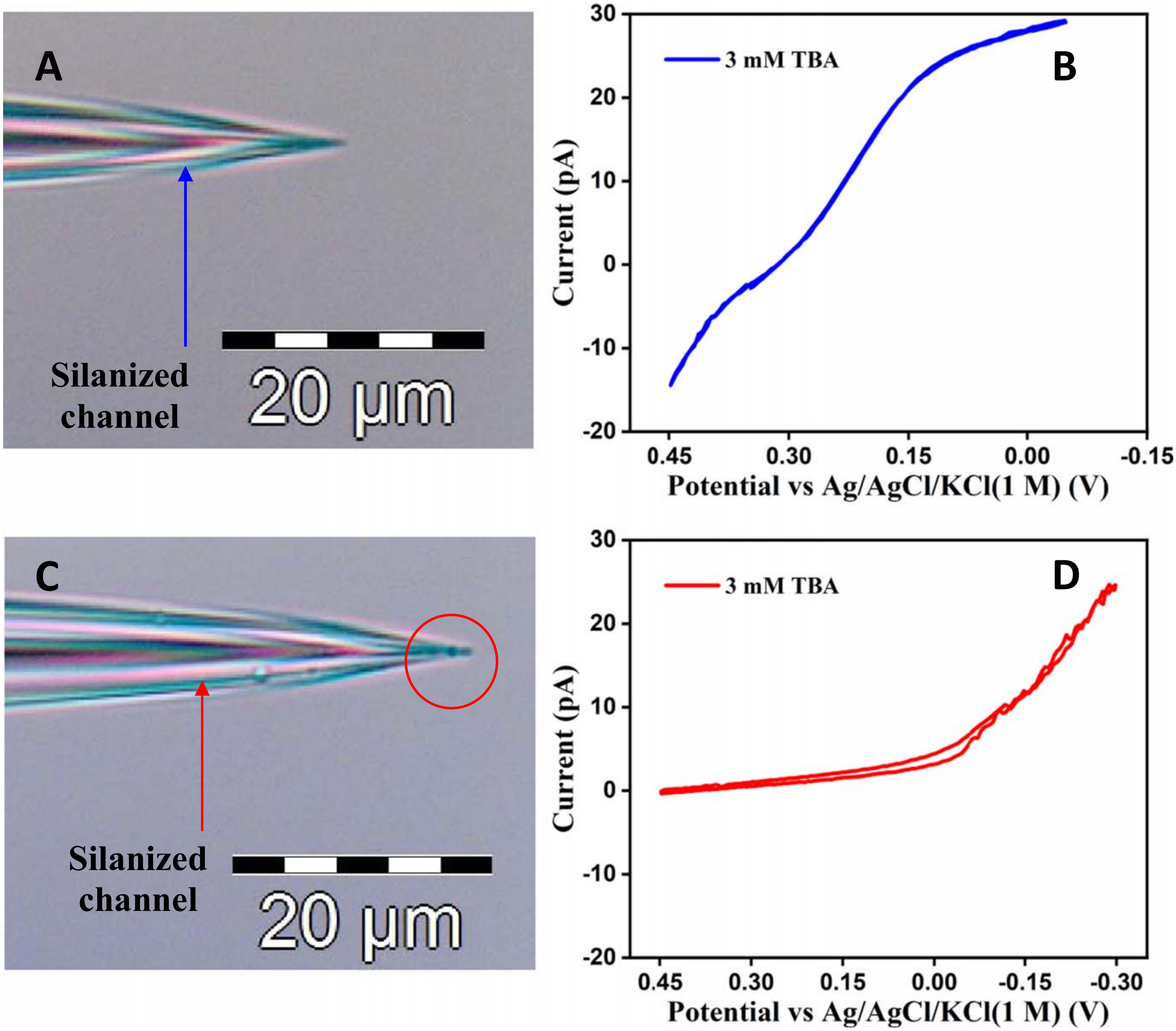

Figure 4. Electrochemical characterization of liquid-silanized nanoITIES electrodes. Microscopic image of the dual channel pipette which was devoid of remnants of TMS-Cl (silane) (A) and with remnants of TMS-Cl (C) (highlighted in red circle) before cyclic voltammetry experiments. Cyclic voltammograms of ∼3 mM TBA in ASW at DCE/water nanoITIES channel of the dual-channel pipette shown in Figs. 4A (B) and 4C (D), respectively.

configuration, maintaining the pipettes in a vertical position with tip pointing downwards for six minutes where gravity kept the area of exposure of silane to the air smaller and slowed the vaporization process. After the six minutes silanization time, silane was removed from the pipette using a syringe. The suction of silane using syringe was not sufficient to remove all the silane, and often small amounts of silane near the tip end were observed.

One of the main challenges of liquid silanization method described above for nanometer-sized pipettes was the drying of TMS-Cl from the nanometer sized tip end. Drying overnight in air was sufficient to dry off the silane in micrometer-sized pipettes,[4] but for nanopipettes, small drop of silane remains at the tip end. We explored several methods of drying including heating of the pipettes, keeping the pipettes in vacuum at elevated temperatures and using a hair dryer to dry the excess silane. After several trials, hot air drying using a hair dryer followed by vacuum drying was found to be the best method. For vacuum drying, pipettes were stored in horizontal configuration under reduced pressure at 37 °C–42 °C for ∼40 h.

We also encountered another challenge which is related to the drying process. During the initial trials after filling the liquid silane, we constantly observed the presence of particle-like substance in the pipette and near tip end after silanization (Fig. 3D). After numerous failures, we observed that these particle-like substance could prevent the drying of the silane inside the nanopipettes. Thus, we investigated the source of these particle-like substance and devised a solution to prevent these.

Experiments to nail down the source of particle-like substance at the nanotip end: corrosion of metal parts of syringe.—Initial hypothesis regarding the source of particles inside the pipette was the presence of particulate matter in the silane itself (TMS-Cl) or the solvent used for cleaning the syringe (acetone). Filtering the TMS-Cl and acetone with 0.22 μm syringe filter didn't solve the problem. As a control experiment, filtered acetone was injected into the pipette and we still observed the particles inside pipette. One important observation during the trouble shooting was that the metal parts of the syringe were changing its color from shiny metallic silver to rusty brown with use. We hypothesized that the corrosion of the metal parts of the syringe by silane, which is highly corrosive, resulted in the particle-like substances observed inside the pipette after silanization. To test the hypothesis, we thoroughly rinsed (~15 times) the metal needle using acetone and wiped the needle and the piston of the syringe with acetone soaked kimwipe between each use. This seems to help to prevent the formation of particle-like substances inside the pipette. However, as time passing by, this method started to lose its effectiveness. We further explored other methods, such as soaking the needle and the piston of the syringe in acetone and storing them at a lower temperature (4 °C) inside refrigerator. This new method lowered the speed of corrosion (Fig. 3C) compared to the one which was stored in atmospheric conditions (Fig. 3B). Using this method to store the needle and syringe, we have produced liquid-silanized nanopipette that has less chance of forming particle-like substance and that can be dried easily after liquid silanization.

Electrochemical characterization of liquid-silanized nanoITIES. The importance of achieving well-dried nanopipette during liquid salinization was further demonstrated by the cyclic voltammetry experiments where comparative studies between fully-dried and partially-dried nanopipettes were carried out. Cyclic voltammetric results of two nanopipettes in which one was devoid of TMS-Cl remnants at the tip end (Fig. 4B) and the one with the remnants (Fig. 4D) are compared in Fig. 4. For the cyclic voltammetric experiments, the ITIES channel of the nanopipette was backfilled with an organic solution of 5 mM TDDATFAB dissolved in DCE (Fig. 1C). The solution was forced to the tip of the nanopipette using gentle vibrations. The ITIES electrode was assembled as detailed in the experimental section. Pipettes were immersed in 3 mM TBACl in ASW and three replicates of cyclic voltammograms were obtained and averaged. ITIES channel of the dual channel pipette which was devoid of TMS-Cl remnants at the tip end (Fig. 4A) resulted in stable sigmoidal voltammogram (Fig. 4B), whereas the one which had the remnants of TMS-Cl (highlighted with red circle in Fig. 4C) did not give steady state response as expected for nanometer-sized electrode (Fig. 4D). These experimental results suggests that the drying of the silane from the nanopipette tip end is essential to obtain a functional ITIES electrode.

Conclusions

We have developed a liquid silanization method to silanize the inner surface of one channel of a dual-channel nanopipette. To the best of our knowledge, this is the first report of liquid silanization for inner surface of nanometer-sized pipettes. We have shown the importance of perfectly dried pipettes (devoid of silane remnants at tip ends) by electrochemical characterization using cyclic voltammetry. Cyclic voltammetry for the transfer of TBA across nanoITIES showed that the nanoITIES electrodes with silane remaining at the tip end did not produce stable cyclic voltammograms. In contrast, the pipettes devoid of silane at the tip end produced cyclic voltammograms expected for nanoelectrodes.

We have observed and addressed several challenges associated with the liquid silanization of nanopipettes, including the drying of remnants of silane from the nanometer-sized tip end of the pipettes. The drying of the nanopipettes was prevented by the presence of particle-like substance in the pipettes after silanization. We nailed down the source of particle-like substance which was resulted from the corrosion of metal parts of syringe by silane. We further developed storage and cleaning methods for the syringe to reduce the speed of corrosion.

Overall, the liquid silanization methods presented here lay the foundation for future development of theta pipette based dual-functional analytical platform where one channel is nanoITIES. Current work is in progress in Shen lab to develop dual-functional nanoscale carbon-ITIES electrodes for detecting both redox active and in-active neurotransmitters.

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