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GABA Detection with Nano-ITIES Pipet Electrode: A New Mechanism, Water/DCE–Octanoic Acid Interface

Nicholas Toshio Iwai, Michelle Kramaric, Daniel Crabbe, Yuanyuan Wei, Ran Chen, and Mei Shen^{*,iD}

Department of Chemistry, University of Illinois at Urbana–Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, United States

Abstract

Interface between two immiscible electrolyte solutions (ITIES) supported on the orifice of a pipet have become a powerful platform to detect a broad range of analytes. We present here the detection of γ -aminobutyric acid (GABA) with the nanoITIES pipet electrodes for the first time. GABA has a net charge of zero in an aqueous solution at pH $\approx$ 7, and it has not previously been detected at ITIES. In this work, we demonstrated GABA detection at ITIES in an aqueous solution at pH $\approx$ 7, where we introduced a novel detection strategy based on “pH modulation from the oil phase”. To the best of our knowledge, this is the first report of such. Current increases linearly with increasing concentrations of GABA, ranging from 0.25 mM to 1.0 mM. The measured half-wave transfer potential of GABA is -0.401 ± 0.010 V ($n = 22$) vs $E_{1/2, \text{TBA}}$. The measured diffusion coefficient for GABA detection at nanoITIES pipet electrode is $6.09 (\pm 0.58) \times 10^{-10}$ m²/s ($n = 5$). Experimental results indicate that protons generated from octanoic acid dissociation in the oil phase do not come out from the oil phase into the aqueous phase; neither were protons produced in the aqueous phase. NanoITIES pipet electrodes with radii of 320–340 nm were used in the current study. This new strategy and knowledge presented here lays the groundwork for the future development of ITIES pipet electrodes, especially for the detection of electrochemically nonredox active analytes.

Graphical abstract

^{*}Corresponding Author, Fax: +1 (217) 265-6290. mshen233@illinois.edu.

ORCID

Mei Shen: 0000-0002-6033-8308

ASSOCIATED CONTENT

Supporting Information

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Parameters used with laser puller in nanopipet preparation; pH of aqueous background solution following the addition of hydrochloric acid and GABA to the background solution (PDF).

Author Contributions

All authors have given approval to the final version of the manuscript.

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Gamma-aminobutyric acid (GABA) is the most predominant inhibitory neurotransmitter in the central nervous system, and it plays a critical role in brain communication and human health. Electrochemical detection of GABA has been challenging as GABA is not electrochemically redox active; thus, it cannot be detected directly on a carbon electrode. Current electrochemical methods on GABA detection require converting GABA into its redox active components, where a chemical reaction with a derivatizing agent is needed prior to GABA detection.^{1–11} The interface between two immiscible electrolyte solutions (ITIES) supported on the orifice of a pipet has become a powerful platform for chemical sensing. Chemical sensing with pipet ITIES electrodes has the advantage of direct detection for nonredox active species without the need for electrode modification. The detection with ITIES pipet electrodes is based on potential driven ion transfer that is related to the structure of each analyte, and the detection potential follows a Nernstian equation.¹² ITIES pipet electrodes have been successfully used to detect a broad range of chemical species,^{13–22} including environmental and biological chemicals, such as heavy metals and neurotransmitters. Several neurotransmitters have been detected such as dopamine^{23–25} and acetylcholine^{26,27} using macro- or microITIES pipet electrodes, as well as acetylcholine using dual pipet electrodes.²⁸ We recently reported the detection of dopamine, acetylcholine, tryptamine, and serotonin with nanopipet electrodes.^{29,30,21} The detection of GABA with pipet ITIES electrodes of any size scale has not been reported. We present here a new strategy (Figure 1c) to enable GABA's detection at ITIES pipet electrodes in an aqueous solution of pH $\approx$ 7 by introducing an organic acid in the organic (oil) phase contained inside the pipet. We demonstrated the assisted ion transfer of GABA using this newly introduced mechanism. To the best of our knowledge, this is the first report of such.

GABA is a zwitterion and its structure was shown in Figure 1. The structure of GABA indicates that it has two functional groups, the amine ($pK_a = 10.22$) and the carboxylic acid ($pK_a = 4.53$) moieties. As shown in Figure 1a, GABA has a deprotonated carboxylic acid and a protonated amine moiety in an aqueous solution at pH = 7, causing GABA to have a net molecular charge of zero. The net charge of zero prevents GABA from being detected at ITIES pipet electrodes via assisted ion transfer by dibenzo-18-crown-6 (DB18C6), a commonly used ionophore in the field of ITIES with a strong affinity for cationic species. We hypothesize that protonated GABA can be detected. By lowering the pH upon detection to be less than the pK_a of GABA's carboxylic acid moiety, e.g. pH = 3, GABA has protonated carboxylic acid and amine moieties, thus possessing a positive charge, with the structure shown in Figure 1b. We added an organic acid, octanoic acid, to the organic filling solution contained inside the nanopipet electrodes, and observed the detection of GABA at

pH = 7. The detection strategy presented here for the first time can be further used to detect a broad range of other zwitterions in an aqueous environment at pH = 7.

EXPERIMENTAL SECTION

Reagents and Apparatus

Magnesium chloride (MgCl_2) was from Amresco (Solon, OH). Dibenzo-18-crown-6 (DB18C6), tetradodecylammonium (TDDA) chloride, tetrabutylammonium chloride (TBACl), 1,2-dichloroethane (DCE), chlorotrimethylsilane, γ -aminobutyric acid (GABA), and acetylcholine were purchased from Sigma-Aldrich (St. Louis, MO). The TFAB salt of TDDA (TDDATFAB) was prepared by metathesis. Ascorbic acid was purchased from Fischer Chemicals (Pittsburgh, PA). Octanoic acid was purchased from the Aldrich Chemical Co., Inc. (Milwaukee, WI). All reagents were used as received, and solutions were prepared using 18.3 M Ω cm deionized water (ELGA, Woodridge, IL).

Nanopipet Electrode Fabrication and Characterization

Detailed nanoITIES pipet electrode fabrication and characterization were reported in recent work.^{21,29,30,32–34} Briefly, the nanopipets were pulled with a P-2000 Laser Puller (Sutter Instruments Novato, CA) using quartz capillaries (1 mm outer diameter, 0.7 mm inner diameter, 7.5–10 cm length). The pulling parameters are listed in Table S1 under the Supporting Information. A scanning electron microscope (SEM) was used to quantitatively determine the inner radii of the nanopipets. Using a high-resolution sputter coater (Quorum Technologies LTD, Kent, U.K.), the nanopipets were coated with a thin film of Au/Pd, and the nanopipet orifices were imaged through high resolution field emission SEM (FEI dual-beam 235, FEI Co., Hillsboro OR) under a 15 kV electron beam. A transmission electron microscope (TEM; Philips CM200, FEI Co., Hillsboro OR) was also used to quantitatively determine the diameter of the nanopipets under a 120 kV electron beam.

Electrochemical Experiments

The detection of GABA with nanoITIES pipet electrodes was studied using cyclic voltammetry. All electrochemical measurements were performed either using a CHI1205B potentiostat (CH Instruments, Austin, TX) or using a CHI760E potentiostat (CH Instruments, Austin, TX). The oil phase that is contained inside the pipet is 1, 2-DCE. The background solution is 10 mM MgCl_2 , which is commonly used in ITIES studies. A Pt wire was inserted inside the nanopipet; an Ag wire (diameter = 250 μm) coated with AgCl (through an oxidation reaction in saturated KCl solution) was placed in the aqueous phase as an outside reference electrode. A two electrode configuration was used as reported previously,^{21,29,30,32–34} where a potential was applied between the inner Pt wire and the outer Ag/AgCl reference electrode. Negative potential is for positive charge transfer from water to oil, seen as positive current. Tetrabutylammonium (TBA) was added at the end of experiments, so GABA transfer potential with respect to the transfer of TBA was reported.

Quantitative Analysis

Details on quantitative analysis were described in our previous work.^{21,29,30} Briefly, the diffusion limited steady state current at nanoITIES pipet electrodes can be expressed as³⁵

$$i = 4xnFDca \quad (1)$$

where i is the steady-state current, x is a function of the quantity $RG = r_g/a$ (r_g and a are outer and inner tip radii, respectively),³⁵ n is the number of transferred charges, F is Faraday's constant, a is the radius of the electrode, D is the diffusion coefficient of the neurotransmitter, and c is the concentration of the neurotransmitter. A proposed disk geometry for the nanopipet tip was used for the calculation. The limit of detection (LOD) was calculated with eq 2:³⁶

$$\text{LOD} = 3s/m \quad (2)$$

where s is the standard deviation of background without GABA present and m is the slope of the calibration curve. The current values from three replicate background CVs at the diffusion limited potential of GABA transfer were used to calculate the standard deviation of the background, as done in our previous study.^{29,30}

RESULTS AND DISCUSSION

GABA Transfer at Water–HCl/1,2-DCE Interface

First, we studied GABA transfer at the water/1,2-DCE interface and did not observe GABA transfer when DB18C6 was present, as well as when DB18C6 was absent in the DCE phase. Considering that DB18C6 has an affinity toward cationic species^{37,38} and GABA has a net charge of zero in an aqueous solution at $\text{pH} \approx 7$, we hypothesized that the protonated form of GABA can likely be transferred at the water/DCE interface when assisted by DB18C6. We tested this hypothesis in an aqueous solution at $\text{pH} \approx 3$, with the cell diagram shown below:

First, we ran cyclic voltammetry (CV) in an aqueous solution of MgCl_2 ($\text{pH} \approx 7$), and the CV result was shown in Figure 2A as the black dotted curve. After that, we added HCl to the MgCl_2 aqueous solution until the pH is ≈ 3 . We ran another CV with the same nanopipet electrode, and the result was shown as the red solid curve in Figure 2A. Compared to the CV in MgCl_2 only, the CV after HCl addition at the negative potential region has an increase in current starting at -0.4 V. This cannot be from the potential drift, since CVs on the positive potential region overlapped before and after HCl addition. We also did not observe an increase in current upon HCl addition when DB18C6 was absent in the DCE phase. Thus, this increase in current after HCl addition is due to assisted ion transfer of H^+ by DB18C6. Following HCl addition to the MgCl_2 solution, we added 0.25 mM GABA to the aqueous phase. We observed an apparent increase in current, shown as the gray solid curve in Figure 2A corresponding to GABA transfer at the water–HCl/DCE interface. Based on the CV,

GABA transfer at the water–HCl/1,2-DCE interface did not reach a plateau, as expected on a nanopipet electrode due to overlapping with H^+ transfer. We further tested if current increases with increasing concentrations of GABA. We added more GABA and ran CVs with the presence of 0.5 mM, 1 mM, and 2 mM GABA. As shown by the results in Figure 2A, increases in GABA detection current with increasing GABA concentrations were observed. Current increases linearly with increasing GABA concentration up to 1 mM, with the calibration curve shown in Figure 2B. At 2 mM and higher concentrations of GABA, the current for GABA transfer at water–HCl/DCE interface deviated from the linearity. A possible reason for this is that the pH of water–HCl phase after 2 mM GABA addition reaches 4.45 (Table S2), which is close to the pK_a of carboxylic acid group of GABA (4.53). These results confirm our hypothesis that protonated GABA can be detected at ITIES pipet electrodes. Our observation is consistent with previous work by Shao et al., where protonated amino acids were detected at the water–HCl (pH = 0–1)/1,2-DCE interface.³⁹ Based on multiple results, the calculated half wave transfer potential for GABA at the water–HCl/1,2-DCE interface, $E_{1/2}$, is -0.381 ± 0.015 versus $E_{1/2,TBA}$ ($n = 21$).

GABA Detection with NanoITIES Pipet Electrodes at Physiological pH: Transfer at Water/DCE–Octanoic Acid Interface

The successful detection of GABA with HCl addition to the aqueous environment confirms the need for GABA's cationic (protonated) form to achieve detection. However, the method presented above is impractical in vivo, because adding HCl to the cellular medium can lead to rapid cellular degradation. The detection should take place at physiological pH to preserve all biological functions while monitoring cellular activity. Here, we propose a new mechanism and demonstrate GABA detection at $pH \approx 7$ with nanoITIES pipet electrodes, where no HCl addition to the aqueous medium was required. In this new mechanism, we added an organic acid, octanoic acid (Figure 3), to the 1,2-DCE phase to enable GABA detection at $pH \approx 7$. The pK_a of octanoic acid is 4.9; in our experiments we added 252 mM octanoic acid into the DCE phase, where the calculated pH based on weak acid dissociation is 2.75. This pH of 2.75 is lower than the pK_a of the carboxylic acid moiety of GABA (4.53). The cell diagram in these experiments is shown below:

Cell 2: Pt|25 mM DB18C6 + 5 mM TDDATFAB + 1, 2 – DCE + 252 mM octanoic acid||10 mM $MgCl_2$ + X mM GABA ($pH \approx 7$)|AgCl|Ag

We first ran the background CV with the nanoITIES pipet electrode, and the result was shown as the black, dashed curve in Figure 4A. We then added 0.25 mM GABA to the aqueous background solution and ran another CV with the same electrode. As shown by the black solid curve in Figure 4A, a well-defined sigmoidal CV was observed corresponding to GABA detection at the water/DCE–octanoic acid interface. GABA detection at the water/DCE–octanoic acid interface have little overlap with the background (Figure 4A), a drastic contrast to GABA detection at the water–HCl/DCE interface where significant overlap with the background was observed (Figure 2A). We further added higher concentrations of GABA (0.5, 0.75, and 1 mM) and ran CVs for each of these concentrations respectively and sequentially. The results were shown in Figure 4A, where an increasing current

corresponding to GABA detection was observed with increasing GABA concentrations. Currents at the diffusion limited transfer potential, as indicated by the arrow in Figure 4A, -0.55 V versus $E_{1/2,\text{TBA}}$, were plotted against GABA concentration, and the calibration curve was shown in Figure 4B. As shown by the calibration curve, the diffusion limited current for GABA detection increases linearly with GABA concentration. The half wave transfer potential, $E_{1/2}$, for GABA detection at the water/DCE–octanoic acid interface is -0.401 ± 0.010 V ($n = 22$) versus $E_{1/2,\text{TBA}}$, which is similar to the value for GABA transfer at the water–HCl/DCE interface. We further calculated the diffusion coefficient for GABA detection at ITIES pipet electrodes using eq 1 as done in our previous work.^{29,30} Based on multiple independent experimental results with different electrodes, the calculated diffusion coefficient for GABA is $6.09 (\pm 0.58) \times 10^{-10}$ m²/s ($n = 10$), which is consistent with literature values.^{40,41} The calculated limit of detection (LOD) is 22.4 μM for GABA at nanoITIES pipet electrode of 320 nm in radius. The above experimental results provide the first demonstration of GABA transfer at ITIES interface in an aqueous solution of $\text{pH} \approx 7$.

One question is if the protons from octanoic acid (OA) dissociation in the oil phase can come out to the aqueous solution or if OA can come out from the oil phase and produce protons in the aqueous phase. If these scenarios were true, a proton transfer wave in MgCl_2 would be observed. As shown in Figure 5, at the water/DCE interface without adding OA, the background CV from MgCl_2 transfer was shown as the black dotted curve, where current starting at ~ -0.5 V represents Mg^{2+} transfer and current starting at ~ 0.15 V represents Cl^- transfer. After adding HCl to the MgCl_2 solution, the CV (red solid curve) indicates a clear proton transfer starting at -0.4 V. After adding OA to the oil phase, the background CV for MgCl_2 (light blue solid curve) on the negative potential region essentially overlapped with the black dotted curve, indicating the presence of Mg^{2+} transfer only and no proton transfer. This demonstrates that protons do not come out from the oil phase into the aqueous phase, nor were protons produced in the aqueous phase. However, octanoate does come out from the oil phase into the aqueous phase. This is because after adding OA to the oil phase, current on the positive potential region started much earlier, at ~ -0.05 V due to octanoate transfer from water to the oil phase, compared to the transfer of Cl^- which started at ~ 0.15 V. In these experiments, all transfer potentials were calibrated with respect to the transfer of TBA, whose transfer at the water–HCl/DCE interface and the water/DCE–OA interface essentially overlap. No observation of proton transfer, as detailed above, indicates that GABA likely gets protonated at the interface between water and DCE and is then transferred across the interface.

We further propose a mechanism for GABA detection via “pH modulation from the oil phase”. Girault et al. have proposed 5 types of reaction mechanisms for assisted ion transfer at the water (W) and oil (O) interface:⁴² analyte (M^{Z+}) diffuses from W to the W/O interface where it complexes with the ionophore (L) to form an ion complex (ML_m^{Z+}) and gets detected (Mechanism A), ML_m^{Z+} diffuses from O to the W/O interface and releases M^{Z+} into W (Mechanism B), ML_m^{Z+} forms at W and transfers across the W/O interface (Mechanism C), M^{Z+} transfers from W to O where it complexes with L to form ML_m^{Z+} (Mechanism D), and M^{Z+} diffuses from O to the W/O interface where it complexes with L from W to form ML_m^{Z+} (Mechanism E), as shown in Figure 6A–E. In our work, GABA, as an analyte, only exists in the water phase initially and the ionophore only exists in the oil phase initially, so

the possible mechanisms for GABA detection are A and D. No GABA transfer was observed without octanoic acid and DB18C6, indicating that GABA does not transfer from W to O. The data from Figure 5 shows that protons do not come out from O to W. Thus, GABA detection cannot be based on mechanism D, where the analyte needs to transfer from W to O. As a result, GABA detection is based on mechanism A with the modification of protonation at the W/O interface. We propose a new mechanism for GABA detection as shown in Figure 6F. Namely, the analyte (GABA) diffuses from W to the W/O interface where it gets protonated and its protonated form (GABA^+H^+) complexes with the ionophore (L) to form an ion complex ($\text{GABA}^+\text{H}^+-\text{L}$) and gets detected (Proposed Mechanism F), where the source of protons is from the organic acid (octanoic acid) dissociation (Figure 6F). The protonation occurs at the interface.

CONCLUSION

We developed a new strategy for electrochemical γ -aminobutyric acid (GABA) detection based on pH modulation from the oil phase. We demonstrated for the first time GABA detection at ITIES pipet electrodes. By integrating an organic acid, octanoic acid, into the oil phase of the ITIES pipet electrode, GABA was successfully detected at biological pH. We showed that protonated GABA can be detected at the water (W)–HCl (pH $\approx$ 3)/DCE (O) interface, and we further demonstrated GABA detection at the W (pH $\approx$ 7)/O–octanoic acid interface. The calculated limit of detection is 22.4 μM for GABA detection at nanoITIES pipet electrodes of 320 nm in radius. Sigmoidal cyclic voltammograms (CV) were observed for GABA detection at both interfaces, where significant overlap with the background was observed for GABA transfer at the W–HCl/O interface compared to at the W/O–octanoic acid interface. Protons generated from octanoic acid dissociation in the oil phase do not come out from the oil phase into the aqueous phase, nor were protons produced in the aqueous phase; no observation of proton transfer indicates that GABA likely gets protonated at the interface between W and O and is then transferred. Octanoate does come out from the oil phase into the aqueous phase, as evidenced by its transfer starting at ~ -0.05 V versus $E_{1/2,\text{TBA}}$. Further development is in progress to apply ITIES pipet electrodes for practical detection of GABA. Nonetheless, the new strategy presented here allows us to design ITIES experiments in an angle distinctive from current existing studies, positioning us one step closer to measure GABA biologically.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Newly introduced detection mechanism based on pH modulation from an organic acid in the oil phase. This mechanism enables the direct electrochemical detection of γ -aminobutyric acid (GABA), an important neurotransmitter and a zwitterion, with nanoITIES pipet electrodes. Chemical structures of GABA at pH ≈ 7 (a) and pH ≈ 3 (b). The $\text{p}K_a$ of the amine and carboxylic acid moieties of GABA are 10.22 and 4.53, respectively.³¹ Without organic acid modulation, GABA is not detected; in contrast, after adding an organic acid, octanoic acid, to the oil phase contained inside the pipet, GABA is detected (c).

Figure 2.

Cyclic voltammograms for GABA detection generated with 0.25 mM-2 mM GABA in Cell 1 ($Y = 1$). We used the current at $E = -0.465$ vs $E_{1/2, \text{TBA}}$, as indicated by the arrow in (A), in the calibration curve in (B). Scan rate: 0.05 V/s.

Octanoic acid: pH modulator from the oil phase

Figure 3.
Structure of octanoic acid.

Figure 4.

Detection of GABA at biological pH with nanoITIES pipet electrodes. Cyclic voltammograms for GABA detection (A), which is based on organic acid modulated assisted transfer of GABA by DB18C6. The electrode has an inner radius of 320 nm based on the outer radii measured by TEM and calculated using $RG = 1.4$. The cell diagram was shown in Cell 2. Insets: TEM view of the nanopipet electrode. We used the current at $E = -0.55$ vs $E_{1/2, TBA}$, as indicated by the arrow in (A), in the calibration curve in (B). Scan rate: 0.05 V/s.

Figure 5.

Cyclic voltammograms of MgCl_2 (black dotted curve), MgCl_2 with addition of HCl at the water/DCE interface (red solid curve), MgCl_2 with addition of HCl and TBA (pink dashed curve) at the water/DCE interface; MgCl_2 (light blue solid curve) and MgCl_2 with addition of TBA (gray solid curve) at the water/DCE–octanoic acid (OA) interface. Scan rate: 0.05 V/s.

Figure 6.

Mechanisms for assisted ion transfer (A–E).⁴² The mechanism we proposed for GABA detection (F): GABA diffuses from the aqueous (W) phase to the W/oil (O) interface where it gets protonated and its protonated form complexes with the ionophore (L) to form an ion complex ($GABAH^+-L$) and gets detected.