

ELECTROCHEMICAL NANOFLUIDIC ASSAYS IN THE ABSENCE OF REFERENCE ELECTRODE

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ABSTRACT

Implementing a reliable reference electrode in miniaturized electrochemical sensors is challenging. Here, we present an alternative approach, based on redox cycling within a nanogap sensor consisting of two parallel electrodes, in which the reference electrode is omitted altogether. We show that on disconnection of the reference electrode, the solution potential floats to a certain value, which is explored theoretically and experimentally in order to quantitatively predict the potential. The obtained results are in good agreement with the theoretically reconstituted results.

KEYWORDS: Reference electrode, Nanofluidics, Redox cycling, Nanogap sensor

INTRODUCTION

The broad applicability of electrochemical sensors for biomedical applications has spurred the development of miniaturized assays, suitable for integration. Implementing an accurate reference electrode is crucial for the stability and accuracy of the sensor. In the past, several groups have worked on reliable and sustainable references; however, miniaturization of reference electrodes is most challenging and so far suffers from several drawbacks [1]. Thus, working without a reference provides an attractive alternative. Such an opportunity is presented here by integrated electrochemical sensor configurations that rely on more than one electrode.

Previously, we have reported electrochemical nanogap sensors consisting of two parallel working electrodes separated by few tens of nanometers as shown in Figure 1a [2]. Redox-active molecules undergo repeated oxidation and reduction as they travel back and forth within the nanochannel, thereby providing a highly amplified electrochemical current at the two electrodes.

The solution potential and the i - V response of the sensor in the absence of a reference depend on the behaviour of both the polarizable electrodes [3]. In Figure 1b, the changed response upon disconnection of the reference electrode is shown. This change in shape is investigated in this work.

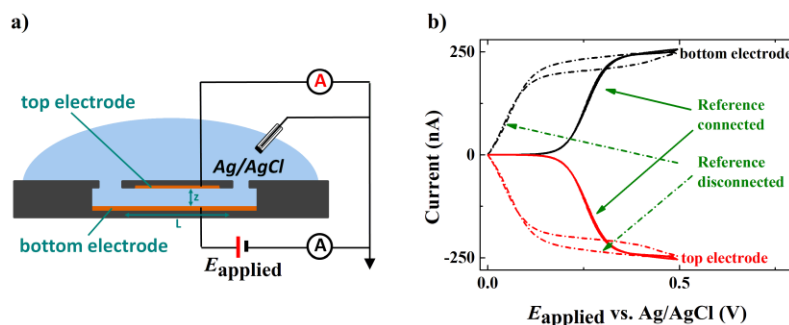


Figure 1: a) Schematic diagram of a nanogap sensor ($z = 65 \pm 5$ nm; $L = 50$ μ m). An Ag/AgCl reference electrode is inserted in the bulk solution and sets the solution potential. b) Cyclic voltammograms showing the redox-cycling response of the nanogap sensor both with and without the reference electrode.

THEORY

In order to understand the changed i - V response of the sensor, we first consider the current response in the presence of a reference electrode at the individual electrodes. Any potential applied to a working electrode is with respect to the solution potential, which in turn is set by the reference. The response at an individual electrode shows a *rest potential* at which the total charge injection of that electrode is zero.

This potential also depends on parasitic currents due to impurities, dissolved oxygen or hydronium ions [2]. On disconnection of the reference electrode, the bulk solution potential is free to drift to this rest potential. However, during redox cycling a known potential is set between two working electrodes, thus preventing the individual rest potentials from being established. Nonetheless, the bulk solution will drift to an (a priori unknown) *equilibrium* potential which is determined by the two working electrodes. As an electrode is swept in cyclic voltammetry, the solution potential shifts appropriately in order to re-establish equilibrium with the electrodes it is in contact with. We directly probed the floating solution potential experimentally to quantify its dependence on the working electrode potentials.

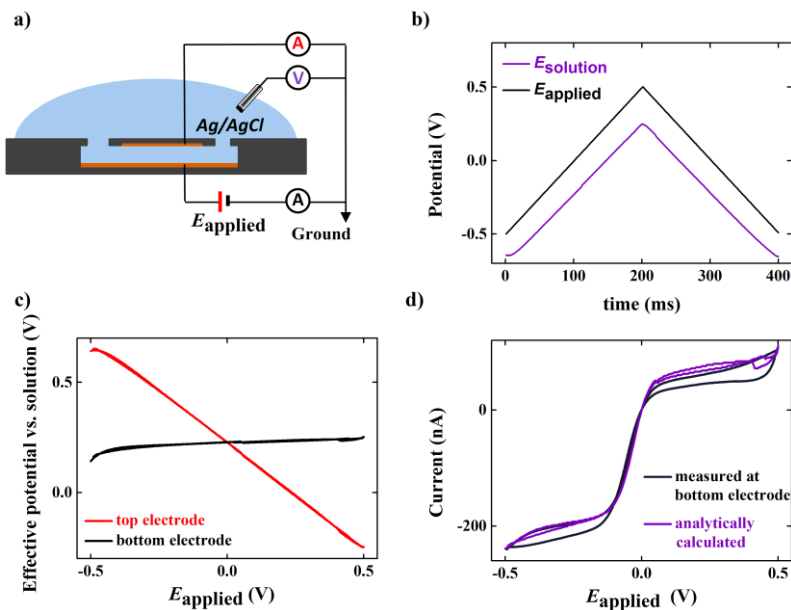


Figure 2: Probing the solution potential during redox cycling without a reference. a) Schematic setup. b) Measurement of solution potential and the applied bottom electrode potential ($-0.5V$ to $0.5V$) during redox cycling (top electrode at $0V$). c) Effective potentials at the top and bottom electrode with respect to the solution potential as a function of applied potential. The solution potential tracks the applied potential of the bottom electrode due to its stronger diffusive coupling to the bulk reservoir outside the device. d) Cyclic voltammograms: the predicted theoretical response using the measured effective potential (Fig. 2c) and the measured voltammogram are shown to be in good agreement.

EXPERIMENTAL

We used nanogap sensors consisting of two parallel planar Pt electrodes separated by a 65 nm gap; they were fabricated as reported previously [4]. A polydimethylsiloxane (PDMS) reservoir filled with analyte solution was positioned above the device and a standard Ag/AgCl reference electrode was inserted in this reservoir. Ferrocenedimethanol $Fc(MeOH)_2$ was chosen as the prototypical redox species and was prepared as a 1 mM solution with 0.1 M KCl as supporting electrolyte.

The response of the sensor in the absence of the reference electrode is shown in Figure 1b. To determine the floating solution potential during redox cycling, we used the Ag/AgCl electrode as a potentiometric probe by connecting it to a high-input-impedance electrometer (Keithley 617) as shown in Figure 2a. The electrode is now a *non-invasive* probe as it is connected to an electrometer and therefore no true reference electrode is employed.

Figure 2b shows the applied potential ($E_{\text{applied}} = -0.5V$ to $0.5V$, black line) with respect to the external ground. The potential of the solution measured (shown in purple) shows that the solution follows the applied potential quite well. Since, in the absence of the reference electrode, the solution acts as the reference (E_{solution}), the effective potential can be calculated as the difference and can be written as

$$E_{\text{effective}} = E_{\text{applied}} - E_{\text{solution}}, \quad (1)$$

where $E_{\text{effective}}$ is the potential difference that is effectively applied to the electrodes with respect to the solution potential. E_{solution} obtained when $E_{\text{applied}} = 0$ V (corresponding to the rest potential) is found to be 0.21 V. During cyclic voltammetry, these observed potentials at the bulk solution were analysed using equation (1) in order to yield the potential difference at each electrode surface (Figure 2c). Thus, oxidising and reducing potentials are observed at bottom and top electrodes respectively when E_{applied} is varied between 0V to 0.5V. We used these data to theoretically reconstitute the expected shape of the redox-cyclic voltammogram at the oxidising electrode. Substituting the measured effective potentials in the kinetic Butler-Volmer equation [2], i.e., the analytically predicted i - V response, leads to a good agreement with the experimentally observed current response in the absence of the reference (Figure 2d).

RESULTS AND DISCUSSION

The above measurements show that, on removal of the reference electrode, the potential to which the solution drifts to is predetermined by the rest potential of zero charge injection which in the case of our experiment is about 0.21 V at each electrode. As E_{applied} is varied, the potential of the solution changes appropriately. The rest potential of the device can be determined by sweeping the individual electrodes. We observe that during redox cycling, when two different potentials are applied to the two separate working electrodes, the influence of the *bottom electrode* on the solution potential is dominating. We attribute this to the specific nanochannel device geometry wherein the bottom electrode lies directly beneath the access holes, i.e., the bottom electrode is more directly exposed to the bulk solution in the reservoir.

CONCLUSION

We conclude that, counter to intuition, the high current levels present during redox cycling do not translate into a higher stability in the absence of a proper reference. Instead, the much smaller currents that flow between the nanofluidic device and the external solution dominate the electrostatic response of the bulk solution. This conclusion holds generally and represents a key insight for the rational design of integrated electrochemical sensors without reference electrodes.

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