

Reference-Electrode Free pH Sensing Using Impedance Spectroscopy †

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† Presented at the Eurosensors 2018 Conference, Graz, Austria, 9–12 September 2018.

Published: 30 November 2018

Abstract: We present a reference-electrode free sensor able to measure both pH and conductivity based on impedance spectroscopy. The electrode is made of a layer of indium-tin-oxide (ITO). The impedance of this electrode at low frequencies depends on its double layer capacity, which varies with pH due to modification of oxide groups at the ITO surface. At high frequencies, the impedance is determined by the resistance in the system, which corresponds to the inverse conductivity of the solution. Because no reference electrode is needed for this technique, miniaturization of the pH sensor is simple. We demonstrate a proof-of-principle experiment of the sensor for human plasma pH measurements.

Keywords: pH sensor; impedance spectroscopy; conductivity; indium tin oxide; double layer capacitance; human plasma

1. Introduction

Knowledge of the pH and conductivity of a solution is relevant for many applications and determinations. In dialysis especially, the acid-base status of the patients' blood is monitored to control the treatment quality. Furthermore, in sorbent dialysis as applied in portable and wearable artificial kidneys, there is a need for incorporation of pH and conductivity sensors to monitor dialysate quality. Since the greatest challenge in developing these artificial kidneys is miniaturization of the device, miniaturization of the sensors is also crucial. While conductivity sensors are generally easy to miniaturize, miniaturization of pH sensors has been an ongoing project for many years. Standard pH electrodes, as well as ion-sensitive field effect transistors (ISFETS) are based on potentiometric techniques and hence require a reference electrode. Stable reference electrodes generally contain an inner electrolyte, which severely limits the options for miniaturization of reference electrodes for extended use. For ISFETS, attempts have been made at fabricating so called REFETS, reference-electrode field effect transistors, but long-term stability of these remains problematic[1].

We have found that by using impedance spectroscopy as the measurement technique instead of potentiometry, we can circumvent the need for a reference electrode. Using indium-tin-oxide (ITO) layers as the sensing element, we have been able to obtain promising results in the determination of pH on artificial buffers, as well as on human plasma. By measuring the impedance at only two frequencies, we sense the pH, as well as the conductivity of our samples.

The principle behind the pH sensing is the change of the ITO surface charge due to (de)protonation of the oxide groups. This is reflected in a change in the double layer. Since impedance

measurements at the right frequency probe the double layer capacitance, they are sensitive to these changes. We are developing a model to theoretically describe our measured data. In this model, we use the equivalent circuit shown in Figure 1 to represent the ITO electrode and sample. The model is based on previous work of our group [2,3].

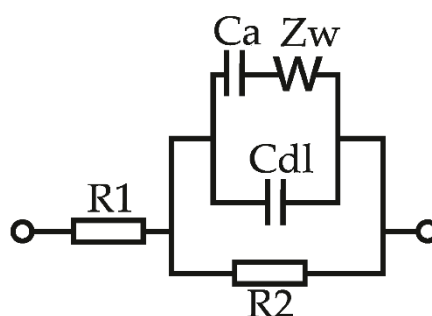


Figure 1. Equivalent circuit used for theoretical modeling of the acquired data. R1 is the electrolyte resistivity, R2 the interfacial resistance, Cdl the diffuse layer capacitance; Ca the capacitance due to adsorbed charge (protons) and Zw a Warburg element representing the diffusion of protons towards the surface.

2. Materials and Methods

2.1. Chemicals

Phosphate buffered saline (PBS) tablets were ordered from Sigma-Aldrich (Zwijndrecht, the Netherlands). Dissolving 1 tablet in 200 mL DI gives 0.01 M phosphate buffer; 0.0027 M KCl; 0.137 M NaCl; pH 7.4 at 25 °C. PBS buffers at various pH values were prepared by adjusting the pH of this standard PBS buffer (original pH 7.4) with Na₂HPO₄ or phosphoric acid. 0.1 M solutions of NaOH and HCl were used to adjust the pH of the human plasma sample. NaOH, HCl (37% solution in water), Na₂HPO₄ and phosphoric acid (≤85 wt % solution in water) were ordered from Sigma-Aldrich and used as received.

2.2. Impedance Measurements

Impedance measurements were performed using a bipotentiostat (SP300, Bio-Logic SAS, Seyssinet-Pariset, France) in a two-electrode configuration. ITO-covered glass slides were ordered from Sigma-Aldrich (Surface resistivity 70 Ω/sq to 100 Ω/sq), and cut to 1 cm × 2 cm. Additionally, three parallel shallow cuts were made in the chips, dividing the ITO into four separate lanes, resulting in four electrodes on one chip. During the measurements, three of these four electrodes were used. To prevent electrolyte leaking from the cell through these cuts, drops of UV-curable glue (NOA 81, Norland, Cranbury, NJ, USA) were positioned on the cuts and the glue was allowed to fill the cuts due to capillary interactions. When the glue in the cuts had entered the sensing area and therefore water tightness was ensured, the glue was cured using an UV light (in-house built, 6 s pulses; 350 mW cm⁻², 365 nm). During measurements, the first and third ITO lanes were connected and used together as counter (and pseudo-reference) electrode; the second ITO lane was used as working electrode. Impedance spectra were measured at the open circuit potential with an AC disturbance of 10 mV. The electrochemical cell used is shown in Figure 2a; an ITO electrode is shown in Figure 2b.

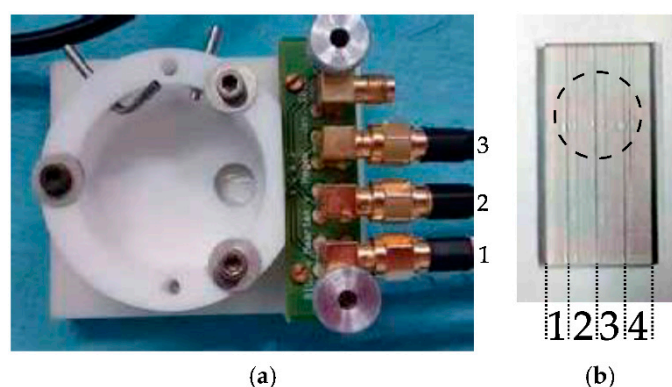


Figure 2. (a) Electrochemical cell used for impedance measurements on buffers. (b) Electrodes as used in measurements. The dashed circle indicates the area that is exposed to the sample. Lanes 1 and 3 were used as counter electrodes; lane 2 was used as working electrode; lane 4 was not used.

From the full spectrum, the frequencies at which the phase was maximal ($\pm 0^\circ$, 300 kHz) and minimal ($\pm 80^\circ$, 10 Hz) were obtained. The impedance at these frequencies was recorded over time while the pH or concentration of buffer in the cell was varied. pH sensing experiments were performed in PBS (0.01 M phosphate buffer). The starting concentration for the conductivity sensing experiments was the same PBS buffer at pH 7.3. During the concentration-variations the pH of this (diluted) buffer varied between 7.3 and 7.5. A commercial pH meter (SevenMulti, Mettler Toledo, Tiel, The Netherlands) was used as a reference to determine the pH of the solutions in the cell. Because the volume of the solution in the cell was too low to use the pH meter directly in the cell, identical manipulations as performed on the solution in the cell were performed on a larger volume of the same solution in a separate vial and the pH of this reference solution was monitored. No reference measurements were performed for the conductivity; only relative concentration and impedance are compared in this work.

Human plasma was obtained from a volunteer after informed consent was provided. The pH of the sample was adjusted using 0.1 M solutions of HCl and NaOH. Two measurements at comparable reference pH values were used to correct for linear drift during the experiment.

3. Results and Discussion

Figure 3 shows the impedances measured at 10 Hz and 300 kHz while varying either the pH or the concentration of a phosphate buffered saline solution. This figure reveals the correlation between the pH or conductivity and the impedance at 10 Hz and 300 kHz, respectively. In Figure 3c,d, the mean plateau value for each plateau is plotted against the corresponding pH or relative concentration and the least squares linear fits are shown. R^2 values of 0.994 and 0.973 indicate the strong linear dependence of impedance on pH and dilution factor. Figure 4 shows the results of impedance measurements on a human plasma sample in which the pH was varied by additions of 0.1 M hydrochloric acid and sodium hydroxide. These data show the applicability of the method for clinically relevant samples. We will further improve the sensor by using temperature control and a closed (flow) cell to avoid escape of CO_2 .

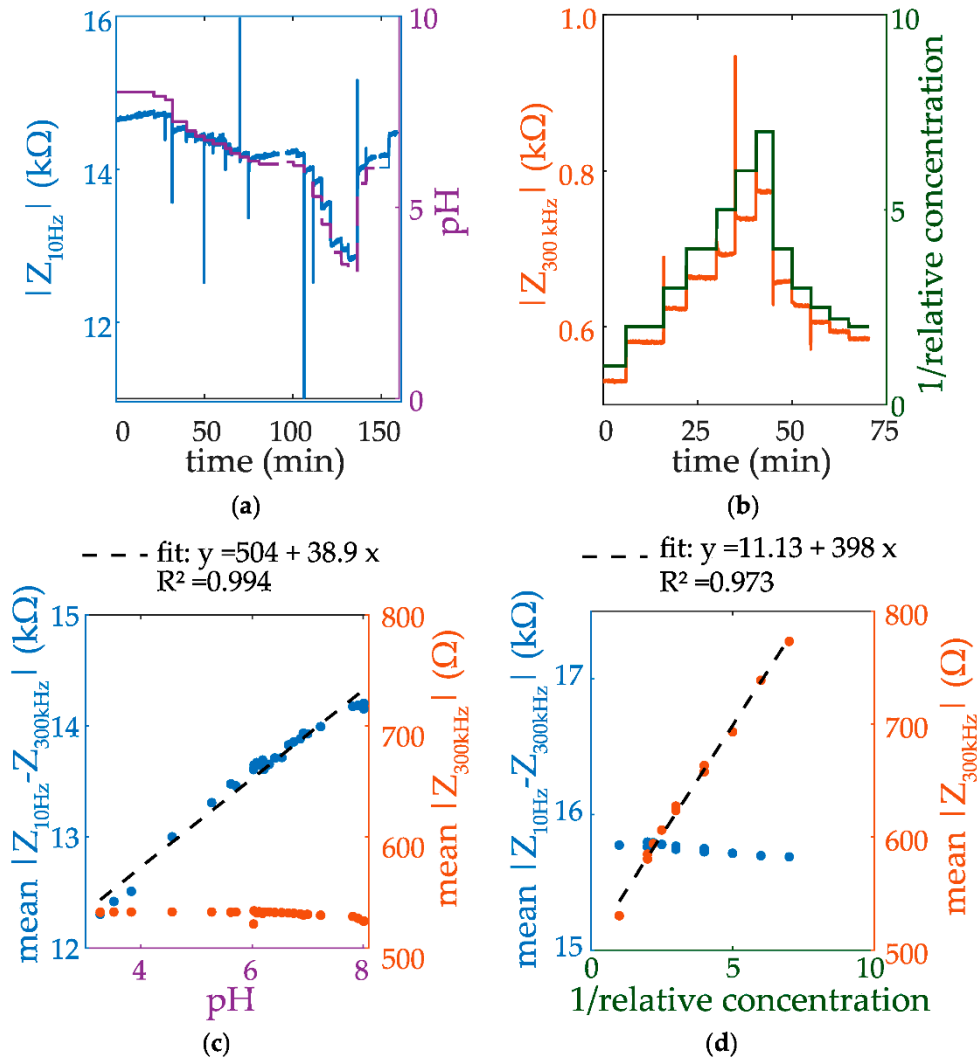


Figure 3. (a) Impedance of the ITO electrode at 10 Hz (blue) and pH (green) vs time. (b) Impedance of the bare ITO electrode at 300 kHz (red) and 1/relative concentration (=dilution factor, green) vs time. (c) Mean $|Z|$ values per applied pH value (blue: 10 Hz–300 kHz; red: 300 kHz). (d) Mean $|Z|$ values per applied dilution factor (blue: 10 Hz–300 kHz; red: 300 kHz).

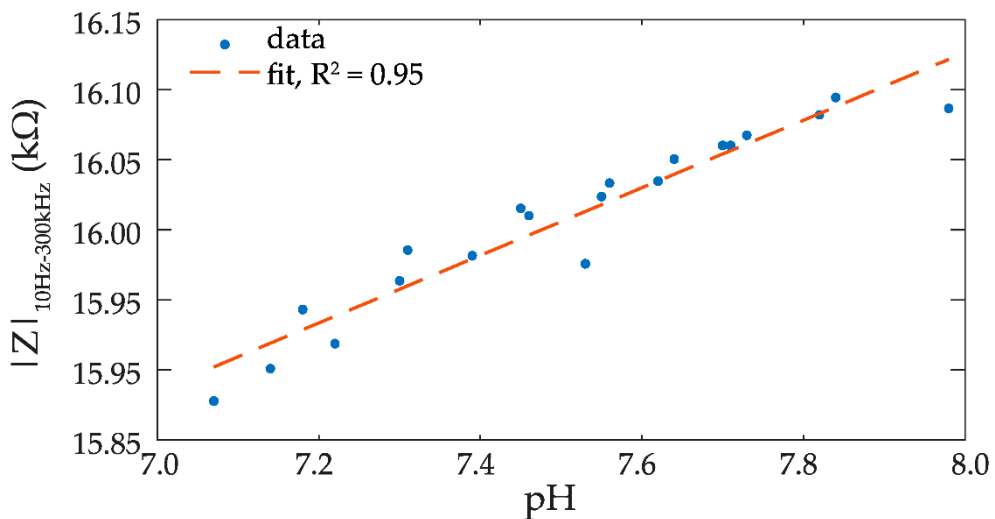


Figure 4. Impedance of the ITO electrode vs pH of a human plasma sample after removal of drift (3 Ω/min). The pH of the plasma sample was varied by additions of aqueous sodium hydroxide and hydrochloric acid (both 0.1 M). Linear least squares fit: $|Z| = 241 \Omega/\text{pH} \cdot \text{pH} + 1.42 \text{ k}\Omega$.

4. Conclusions

Impedance measurements on ITO electrodes are a promising technique to simultaneously measure pH and conductivity, without need for a reference electrode. This means that the combined pH and conductivity sensor will be easy to miniaturize. The resulting miniaturized sensor could be a solution for pH and conductivity sensing in flow-through applications, such as the portable artificial kidney.

Acknowledgments: This work was funded by project number 728.011.205 from the research programme “NWO ChemThem Out-of-Equilibrium Self-Assembly” and by the financial support of the strategic alliance of the Technical Medical Centre (formerly MIRA institute) of the University of Twente, the University of Utrecht and the University Medical Center Utrecht. LG received a scholarship from the Erasmus Programme.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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