

Supported BLM Microprobe with Thin-Film Ag/AgCl Reference Electrode for pH Measurements

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A measuring “microcell” consisting of a thin-film Ag/AgCl reference electrode and a bilayer lipid membrane-based microprobe on a thin-film metal support is developed. We study the influence of the thin-film supports (platinum or gold) and the pH-sensitive mediating supplements tetracyano-p-quinodimethane (TCNQ) or tetrachloro-m,o,p-benzoquinone (TCmopBQ) of the sl-BLM membrane-forming lipid solution on the electrical properties (capacitance and conductance) and the pH response of the membrane. Additionally, the dependence of the essential Ag/AgCl electrode parameters on the conditions under which it is prepared are shown.

1. Introduction

In general, electrochemical sensors are one of the largest groups of chemical sensors and have many applications in the chemical, environmental and medical industries. The need for miniaturization and mass production of electrochemical sensors has increased the importance of microsystem technologies. Thin-film and IC technologies have been successfully utilized in the development of planar electrochemical micro-sensors.

All biological membranes are based on self-assembled bimolecular lipid layers, i.e., the bilayer lipid membrane (BLM). These membranes, and especially a self-assembled BLM on a solid support (s-BLM), show much promise as a system for lipid-bilayer-based electrochemical sensors. Solving the problem of the compatibility of BLMs with planar

microsystems is essential for their future application in the field of microbiosensors and bioelectronics. Although previously created under laboratory conditions in the early 1960s,⁽¹⁾ to date, BLMs have not been applied for sensor design due to their poor mechanical stability and incompatibility with planar technologies (aqueous solution/BLM/aqueous solution). Despite these drawbacks, they have become an important experimental tool of membrane research. Suitably modified, these membranes serve as highly selective and sensitive transducers, often with extremely low detection limits.⁽²⁾ Moreover, BLMs make up a natural environment for various biological receptors and enzymes, which makes them attractive candidates for use in biosensors and bioelectronic devices.

A major breakthrough was achieved in the late 1980s and early 90s with the invention of the supported BLM (s-BLM).⁽³⁻⁵⁾ Unlike a conventional BLM that separates two aqueous solutions, the s-BLM is created on the cross section of a freshly cut metal wire⁽³⁾ or on a thin-film metal deposit surface (lipids can also be attached to a gold surface via polar linkers and sulfur-gold bonds⁽⁶⁾). In the latter case (marked as sl-BLM), the surface properties of the support can be better controlled by setting proper parameters for the deposition procedure. Namely, it is assumed that a microscopically rough metal surface causes local disturbances in the membrane structure and, consequently, leaks. The membrane conductance can therefore be 10 to 100 times higher than in the case of conventional BLMs.⁽⁷⁾ Both s-BLM and sl-BLM exhibit a highly asymmetrical arrangement (metal/BLM/aqueous solution) as compared with the conventional BLM, which is why there are considerable differences in their electrical and mechanical properties. However, much better mechanical stability is reached in the case of supported BLMs, and at the same time, the necessary planar "bioelectronic" interface is created.

The parameters utilized most frequently to evaluate the BLMs are i) electrical parameters such as membrane potential (E_m), capacitance (C_m), conductance (G_m), and breakdown voltage and ii) mechanical parameters such as elasticity modulus and viscosity. These parameters are dependent on the kind of lipid, solvent, mediator (its type and concentration), temperature and (in the case of supported BLMs) the support parameters.

In our previous paper⁽⁸⁾ we analyzed the dependence of the electromechanical properties (elasticity modulus and capacitance) for supports having considerably different degrees of roughness (s-BLM on a cross section of a cut wire and sl-BLM on a thin-film support). We concluded that the considerably higher surface roughness in the case of the s-BLM made the membrane inhomogeneous and, as a consequence, relatively high dispersions of the capacitance and elasticity modulus were observed. Thin-film supports with different degrees of roughness and properties controlled by modifying the parameters of the deposition procedure, could be prepared.

To obtain a reliable response from a sensor based on a supported BLM (and in general, for any electrochemical sensor) the reference electrode is of crucial importance. Since the potential of the reference electrode is a known constant under all circumstances, changes in the potential are only related to variations in the potential at the working (for example, sl-BLM) electrode. Therefore, the reference electrodes are of special interest for the development of electrochemical sensors. Other kinds of reference electrodes, such as Ag/AgCl/Cl⁻, are used in sensor planar microsystems.^(9,10) In many applications they can be operated as pseudoreference Ag/AgCl electrodes. For example, in amperometric-based

sensors, small deviations of the reference electrode potential have no influence on the response of the sensor because the current is recorded at the current plateau. On the other hand, for potentiometric sensors, well-defined reference electrodes are desirable (Ag/AgCl/Cl⁻). In terms of planar constructions, this means that the problem of "inner" electrolyte encapsulation with sufficient and constant Cl⁻ concentration must be solved. This can be realized, *e.g.*, by using polymeric conductive electrolytes.⁽¹¹⁻¹³⁾ Important requirements for planar reference electrodes are the following: (i) stability of the potential, (ii) reproducibility and repeatability of the potential, (iii) long lifetime (corrosion resistivity), (iv) temperature stability, and (v) low noise.

In this paper we report on the development of a Ag/AgCl-based planar thin-film reference electrode, as well as the development of BLMs supported by thin-film metal electrodes (sl-BLMs) used for pH measurements.

The idea of using BLMs in pH sensors is based upon the fact that biological membranes are essentially natural sensors that selectively transport ions and/or electrons through the membrane. Detection in a biological environment must be carried out using a sensing element that is biocompatible.

Although commercially available ISFET sensors for detecting pH are, from the viewpoint of their stability and practical applicability, superior to that proposed here, the pH-sensitive sl-BLMs can be used for indirect detection of biomolecules in enzyme sensors.⁽⁹⁾

2. Materials and Methods

Thin films for both the reference and the working (with sl-BLMs) electrode were prepared on silicon/silicon dioxide wafers (3 inches in diameter) by r. f. sputtering and photolithographic patterning.

For the reference electrodes, the silver (Ag) layer with a thickness of 1,000 nm and the underlying adhesion and barrier layers (Ti/Pd) were prepared and patterned. Polyimide (Brewer Science) or photoresist (Hoechst) was used as a protective film with a rectangular window (area: 1.85 mm²) for Ag chloridation (Fig. 1). The anodic chloridation of the Ag layer was carried out in 0.01 mol/l KCl electrolyte at 700 mV with respect to the saturated calomel electrode (SCE) using a potentiostat unit (Radelkis OH 404 type). The total chloridation time (the time needed for full chloridation of the Ag layer) was about 60 s. Three different chloridation times were used for AgCl preparation (15, 30 and 60% of the total chloridation time) to study the effect of different AgCl/Ag thickness ratios on the stability of the electrode potential. To convert the pseudoreference electrode into a reference one, a gel layer (2% agar in a 0.1 mol/l KCl solution) was applied on top of the Ag/AgCl layer. The electrode potential was measured using a HP 34401A multimeter with a sampling time of 1 s or 1 ms, versus the SCE using a salt bridge and a thermostated solution. The solution was not buffered.

The working electrode consisted of a platinum (Pt) or gold (Au) layer with a thickness of 150 nm with the same underlying adhesion and barrier layers (Ti/Pd). Furthermore, the same protective film with a circular window for sl-BLM preparation (area: 0.36 mm²) was used (Fig. 2). The sl-BLMs were self-assembled in the aqueous phase (0.1 mol/l KCl) in

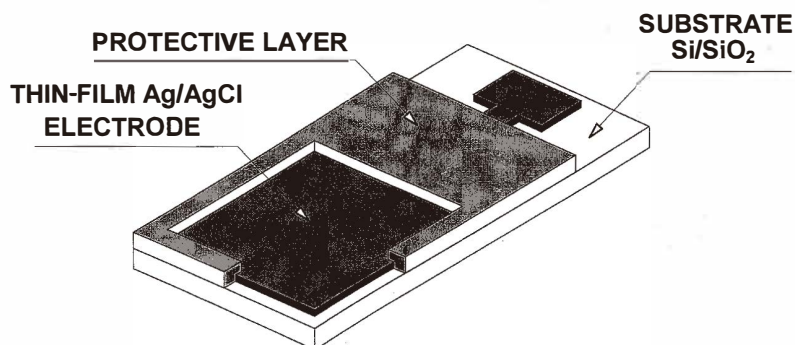


Fig. 1. Design of the thin-film Ag/AgCl reference electrode.

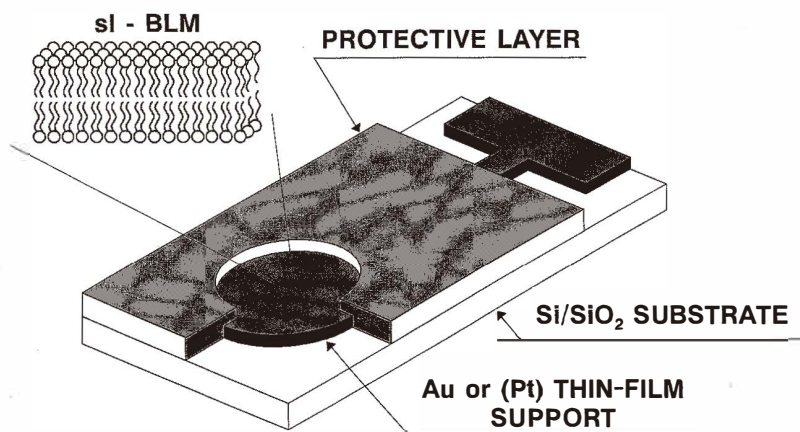


Fig. 2. Design of the thin-film metal electrode used for sl-BLMs preparations.

two subsequent steps: i) application of the membrane-forming lipid solution on the metal surface in the window and, after a certain period of time ("preconditioning"), ii) immersion of the support containing the lipid solution into an aqueous phase ("forming") (see ref. (3) for more details). Asolectin (Serva) dissolved in *n*-decane (Merck; 50 mg/ml) was used as a membrane-forming lipid solution. To induce pH sensitivity in the BLM, the lipid solution was saturated with pH-sensitive mediators (TCNQ or TCmopBQ). To measure the pH response, the aqueous solution was buffered to pH values ranging from 4 to 8 using a universal Britton-Robinson buffer.

The on-going formation of the sl-BLM starting with the immersion of the lipid-treated support into the aqueous solution was evaluated using cyclic voltammetry (CV). The same

method was used to obtain the electrical parameters, i.e., membrane capacitance (C_m) and conductance (G_m). Steady-state C_m and G_m values indicated that a bilayer had formed. The cyclic voltammograms were recorded under the following conditions: polarizing potential with a triangular waveform from -100 to 100 mV vs SCE and a scan rate of 10 mV/s.

The pH sensitivity of the modified sl-BLMs was evaluated potentiometrically using a simple portable pH meter/voltmeter with a universal input adapted to different kinds of pH-sensitive or ion-selective electrodes (input impedance better than 10^{11} ohm) designed and constructed in our laboratory.

Both CV and potentiometric measurements were carried out in an arrangement using a salt bridge. As a reference electrode, a thin-film Ag/AgCl or a saturated calomel electrode (SCE) was used.

3. Results and Discussion

3.1 Thin-film planar Ag/AgCl reference electrode

3.1.1 Stability, reproducibility and repeatability of the Ag/AgCl electrode potential

In general, the most important features of a reference electrode are stability of the potential, reproducibility and repeatability, a long lifetime, temperature stability and low noise level.

To optimize potential stability and reproducibility, electrodes with different AgCl/Ag thickness ratios were prepared. The best results were achieved with electrodes whose thickness ratios corresponded to 30% of the total chloridation time. Electrodes prepared in this way were used in all of the following experiments. Significant results were obtained by recording the response of the pseudoreference electrode immediately after its immersion into the electrolyte. Figure 3 shows a typical potential vs time chart. Good visible "undulation" typical for all samples was caused by convection in the solution. However, after a period of about 1s the potential reached a steady-state value. The average value for the pseudoreference electrode was 45.9 mV vs SCE measured 1 min after its immersion into the 0.1 mol/l KCl solution at 25°C . Under the above-mentioned conditions, the potential should be 46 mV vs SCE.⁽¹⁴⁾ However, a small negative drift of potential with time was observed (-3 $\mu\text{V/s}$). We assume that this phenomenon is probably due to AgCl dissolution in the electrolyte.



In accordance with the Nernst equation, the potential is reduced if the chloride ion concentration increases. The potential of the reference electrode with the agar gel layer vs SCE is lower (44.5 mV) and a positive drift is observed in this case (1 $\mu\text{V/s}$; Fig. 4). The contradiction with the above-mentioned result can be explained by the influence of the agar gel layer. It seems, namely, that the Cl^- ion concentration in the agar gel is higher than 0.1 mol/l due to water evaporation during the gel preparation. Therefore, the potential drift has a positive value until the Cl^- ion concentration in agar is balanced by diffusion with 0.1 mol/l Cl^- concentration in the measuring solution. Long-term records of the potential stability confirmed our assumptions.

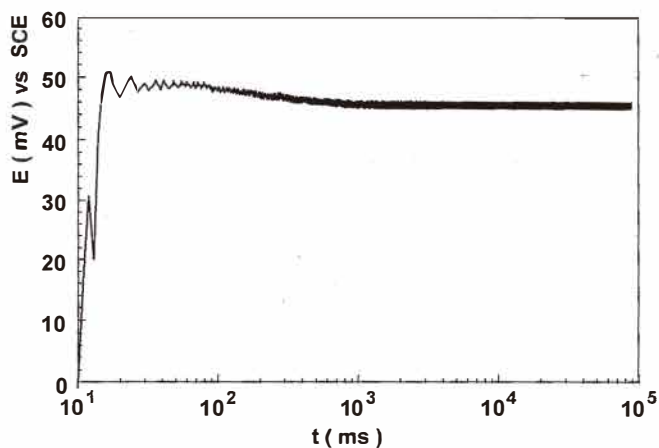


Fig. 3. Development of the thin-film Ag/AgCl pseudoreference electrode potential (measured vs SCE) at 25°C in 0.1 mol/l KCl solution.

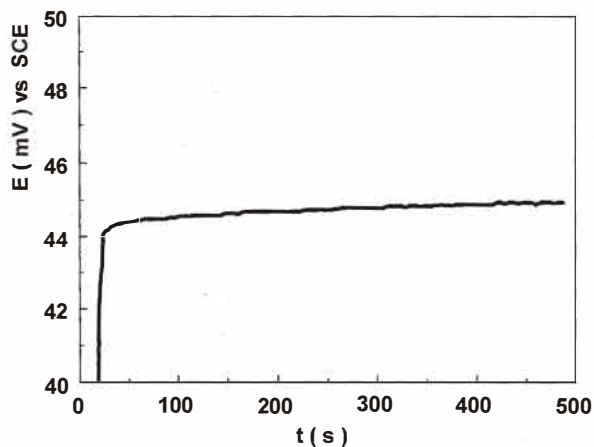


Fig. 4. Development of the potential of the thin-film Ag/AgCl reference electrode covered by an agar gel layer (2% agar with 0.1 mol/l KCl). Recorded vs SCE at 25°C in 0.1 mol/l KCl solution.

One very important parameter of reference electrodes is the reproducibility of the potential for multiple use. Potential values measured over a 5-day period for pseudoreference and reference electrodes were dispersed around their average values by not more than $\pm 1.2\%$ and $\pm 0.9\%$, respectively.

3.1.2 *Ag/AgCl electrode potential dependence on the chloride concentration*

When applying Ag/AgCl electrodes to electrochemical sensors, the influence of Cl^- ions on the potential is very important. According to the Nernst equation the dependence of potential on the Cl^- concentration is logarithmic. Figure 5 shows the potential dependence on the Cl^- concentration of the pseudoreference and reference electrodes. While the dependence of the pseudoreference electrode shows Nernstian behavior, the potential of the reference electrode is 46.3 ± 1.1 mV vs SCE for the Cl^- concentration range of 0.01 to 0.4 mol/l, i.e., it is practically independent of the chloride ion concentration. For the application in clinical diagnostic biosensors where the analytes are biological fluids with a practically constant Cl^- concentration (150 ± 9 mmol/l), such as blood,^(9,14) this change in the potential should be negligible.

3.1.3 *Temperature dependence of the Ag/AgCl electrode potential*

The temperature dependence of the Ag/AgCl electrode potential was tested from 20 to 40°C. To eliminate the influence of the temperature response of the SCE (0.55 mV/°C) on the measured value, two vessels connected by a salt bridge were used. One of them (with the SCE) was held at a constant temperature of 25°C, while the temperature of the second one (with the Ag/AgCl electrode) could be set to desired values. For the above-mentioned interval, the temperature coefficient was 0.3 mV/°C. This coincides with the value presented in ref. (14). The authors also pointed out that this value is higher than assumed, which indicates some nonstationary state of the silver electrode interface.

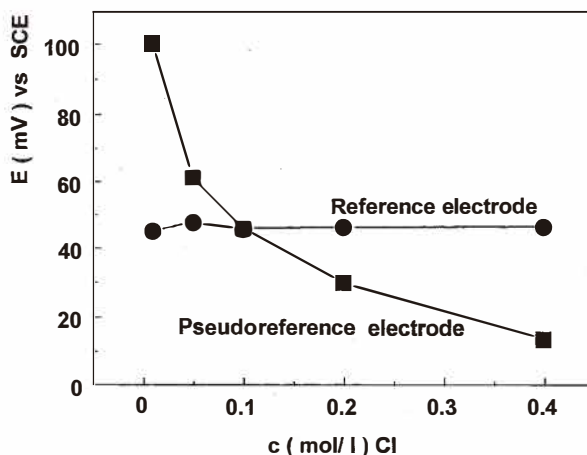


Fig. 5. Dependence of the potential on the Cl^- concentration in aqueous solution in the case of pseudoreference and reference electrodes.

3.2 pH-sensitive microprobe based on sl-BLM

3.2.1 Cyclic voltammetry of the sl-BLMs

The aim of these experiments was to determine the effect of a thin-film support on the electrical parameters (C_m , G_m) of sl-BLMs. Most of the measurements show better mechanical stability and a particularly reproducible potential of sl-BLMs with gold supports compared to those with platinum supports. We assume that this is related to a higher oxidation rate of the platinum surface in air compared to gold. To confirm our prediction we attempted to recover the platinum surface using a redox procedure in a sulfuric acid solution (for details see ref. (15)) immediately before the application of the lipid solution. After that pretreatment, capacitance and conductance changed less than before. It is difficult to avoid surface oxidation completely because the metal surface should be clean and dry before the self-assembling process of the sl-BLM. However, the supports were exposed to air only for a few seconds before the lipid solution was applied. Typical parameters of the sl-BLMs on different surfaces are summarized in Table 1. The lifetime of the sl-BLMs was typically at least 24 h.

3.2.2 Potentiometry of the sl-BLMs

The sl-BLMs were self-assembled on gold and platinum electrodes. This procedure consisted of two steps. In the first step, the lipid solution was applied to the metal surface. After a certain period of time, the "preconditioning" time, the second step followed, whereby the lipid-treated support was immersed in the aqueous solution upon which the self-assembly process eventually resulted in the formation of a bimolecular arrangement of the lipid molecules on the metal surface. The preconditioning time is critical in terms of obtaining a sl-BLM with high pH sensitivity, when the membrane-forming lipid solution is modified by TCNQ or TCmopBQ, as in our case. The pH-sensitive mediating substances

Table 1
Typical parameters of the sl-BLMs (C_m and G_m) on different thin-film supports.

Support	C_m [$\mu\text{F}/\text{cm}^2$]	G_m [S/cm^2]
Pt (without pretreatment)		
after 10 min	0.28	1.1×10^{-8}
30 min	0.23	1.0×10^{-7}
Pt (recovered)		
after 10 min	0.27	1.4×10^{-8}
30 min	0.25	5.3×10^{-8}
Au (without pretreatment)		
after 10 min	0.20	7.7×10^{-9}
30 min	0.20	7.7×10^{-9}

TCNQ or TCmopBQ probably create channellike structures in the BLM (a process which requires a certain amount of time) which (certainly in the case of TCNQ⁽¹⁶⁾) can be accessed by electrons. As obvious from Figs. 6 and 7, the dependence of the pH sensitivity of the lipid membrane on the preconditioning time confirms this prediction. In both cases,

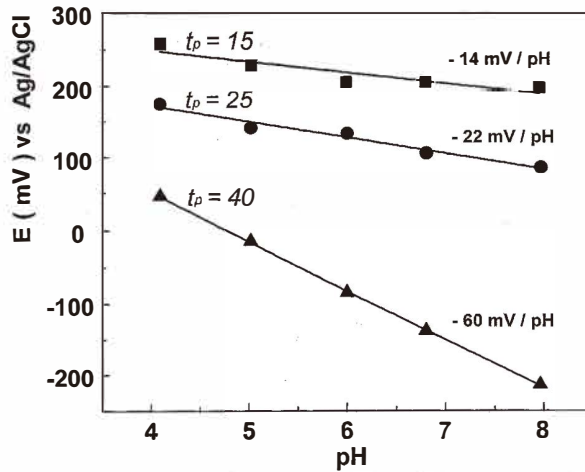


Fig. 6. Dependence of the membrane potential on pH for sl-BLM modified by TCNQ for different preconditioning times.

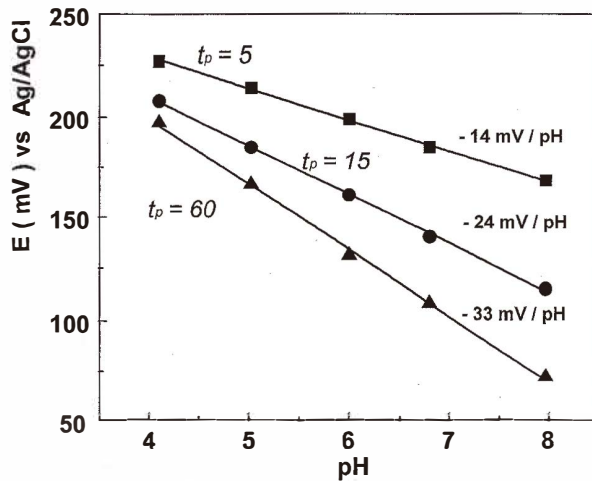


Fig. 7. Dependence of the membrane potential on pH for sl-BLMs modified by TCmopBQ for different preconditioning times.

the same "forming" time was used (10 min). In the case of the highest obtained sensitivity (-60 mV/pH) using TCNQ, the large number of "channels" in the membrane structure unfortunately affected the membrane stability, as indicated by the increased potential dispersion. However, the reason for the lower response of the sl-BLM modified by TCmopBQ is not known at this time. We assume that the electron-conducting performance of the TCmopBQ channellike structures is less efficient.

On the other hand, no influence of the second forming step (starting with the immersion of the lipid-treated support into the aqueous solution) on the sensitivity was observed in intervals from 10 to 60 min. In all cases, the process of sl-BLM formation was completed relatively quickly (within 5 to 10 min).

The sensitivity of an unmodified sl-BLM was found to be -13.5 mV/pH, which is naturally independent of the preconditioning time.

The calibration curves for both modified and unmodified sl-BLM probes are shown in Fig. 8.

4. Conclusions

The microcell outlined in this paper consists of a thin-film microprobe based on a sl-BLM and a thin-film Ag/AgCl reference electrode. Proper parameters have been found for the preparation of thin-film Ag/AgCl electrodes by anodic chloridation, in terms of potential stability, reproducibility and repeatability. The influence of the Cl^- ion concentration in the aqueous solution and of temperature on the potential were studied. The pH-

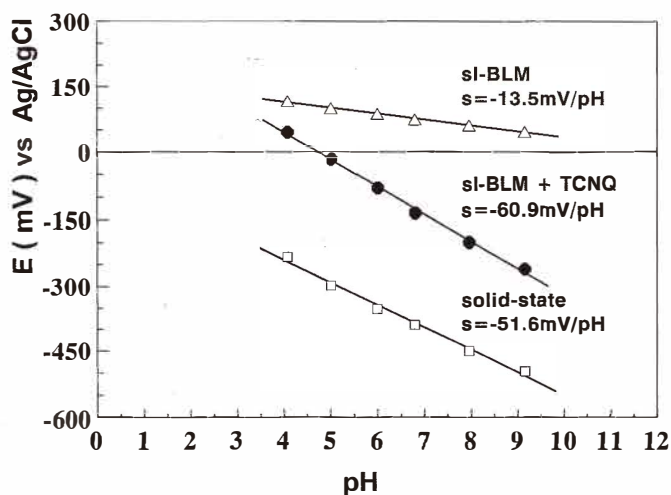


Fig. 8. pH calibration curves for different pH probes.

sensitive microprobes based on sl-BLM (on platinum or gold thin-film supports) modified by TCNQ or TCmopBQ were tested using cyclic voltammetry and potentiometry in a pH range from 4 to 8.

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