Hybrid Organic-Inorganic Biosensor for Ammonia Operating under Harsh Physiological Conditions

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A biosensor for ammonia is developed aimed at detecting the presence of *H. pylori* bacteria in gastric fluids. The sensor is based on a GaAs device coated with a unique functional polymer that enables high device sensitivity to low concentrations of ammonia and long-term protection in harsh environments. The detection of ammonia in gastric fluids taken from patients is possible by covering the device with a dialysis membrane, thus enabling the diffusion of only small molecules to the sensing area, while preventing agglomeration of macromolecules on the surface of the device. The mechanism by which ammonia is detected is investigated and an analytical expression is provided relating the response of the detector to the ammonia concentration and the pH of the solution.

1. Introduction

Detection of ammonia in gases and liquids is of great importance in industrial and environmental applications but even more so in the human body because ammonia is one of the main metabolites. Various types of ammonia sensors were developed and some are even commercially available.[1–5] Specifically, solid-state biosensors, based on microelectronic technologies, were suggested for diagnostic and analytical instruments[6,7] mainly due to their low cost and the ability to produce integrated sensors with many sensing elements.[8–12] However, despite the existence of microelectrodes[13] and sensors based on field effect transistors (e.g., ISFET)[14], the penetration of these technologies suffers from their low sensitivities and instability in harsh physiological environments. The main difficulty in semiconductor-based devices, particularly devices that are based on GaAs, is their corrosion in aqueous solutions. They are chemically and electrically unstable and they release bio-incompatible substances. Therefore, it is difficult to apply them as biosensors in general and even more so as sensors operated in vivo.

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There are a number of diseases associated with abnormal concentrations of ammonia such as Reye's syndrome,[15] hyperammonia,[16] stomach and gastric cancers,[17,18] and ulcers.[19] Among these, stomach cancer and ulcers are known to be caused by a *Helicobacter pylori* (*H. pylori*) bacterial infection. *H. pylori* bacteria reside in the lining of the stomach and release large quantities of ammonia in order to neutralize the local acidity.[20,21] The current clinical analyses that are used to measure the presence of *H. pylori* are breathing tests along with gastric fluid extractions and biopsies,[22] but these procedures are quite tedious and time consuming. Various types of sensors were proposed for direct ammonia sensing; however, they suffer from several drawbacks. For example, sensors based on potentiometric measurements or impedance lose their sensitivities because of the applied over potential.[23–26] The use of enzymatic-ISFETs was also considered but the enzymes are known to interfere with the sensing area, and the drift in the response decreases their sensitivity.[27]

The molecular controlled semiconductor resistor (MOCSER) sensor used in this study was based on a previously reported GaAs device.[28] In the past, the MOCSER-based devices were successfully applied for sensing both in gas[28,29] and in liquid phases.[30–32] The ammonia sensor that we present operates in highly acidic gastric fluids (down to pH 1). The ability to work in this environment was achieved by protecting the semiconductor device with an ultra-thin polymer layer that exposes functional groups towards the analytes. The sensor can operate for hours within the gastro fluid with high sensitivity and detects ammonia down to concentrations of a few parts per million (ppm). It combines two sensing elements that exhibit different response-to-concentration dependences. We provide a proof of concept for the mechanism by which ammonia is sensed and demonstrate the ability to monitor minute concentrations of ammonia without the need to pre-calibrate the device. Hence, the proposed device is unique in its potential to monitor ammonia either ex vivo, in a bench-top apparatus, or in vivo, namely, within the gastro system.

2. Fabrication of the MOCSER Sensor

There are many reports on surface passivation techniques for GaAs; however, most of them fail to provide protection for

prolonged measurements in aqueous environments. An ultrathin polymer coating, chemically deposited by a sol-gel process with 3-mercaptopropyl trimethoxy silane (MPTMS), was shown to significantly increase the long-term stability of GaAs surfaces.[33] Thus, we have adopted this basic concept for the current study, yet we have modified this procedure to create an even more robust polymer coating capable of effectively transferring the potential changes on its surface to the underlying GaAs substrate.[33]

Prior to the molecular coating, the GaAs substrate was etched with hydrofluoric acid and ammonium hydroxide in order to remove the oxide layer and to expose an arsenic-rich surface. The MPTMS molecules were first adsorbed as a monolayer, by binding to the substrate through their thiol ends. Next, ammonium hydroxide (serving as a condensation agent) was added to the solution, inducing MPTMS polymerization and the formation of a dense polymer layer. The resulting polymer layer thickness was about 15–18 nm (with a dielectric constant of about 2.5), as measured by ellipsometry. This polymer coating was found to be stable for more than a week in deionized water and no increase in the oxide thickness on the GaAs surface was observed (as described in ref. [33]). Lastly, a 3-aminopropyl trimethoxy silane (APTMS) layer was deposited on top of the MPTMS layer for further surface modification (see **Figure 1** and Supporting Information). A semi-permeable cellulose-based dialysis membrane with a 10 kDa cut-off molecular weight (Sigma-Aldrich, D9777) was used as a filter to eliminate the arrival of macromolecules on to the device's surface. Eventually, a polydimethylsiloxane (PDMS)-based fluidic system, with an internal volume of 25 µl, was constructed on top of the MOCSER device in order to supply the analyte solutions and allow controllable retention times (see Figure 1).

Ammonia was dissolved in solutions having different acidity and in physiological fluids, and the samples were injected sequentially through the flow cell. Gastric simulation fluids were made by dissolving 3.2 g of pepsin enzyme, 2 g NaCl, and 7 ml of HCl, in 1 liter of water. A syringe pump (Harvard Instruments, PHD) was used to ensure a controllable flow of precise volumes of analytes. Electrical measurements were performed on wire-bonded devices using a dual-channel source-measure unit (Keithley, 2636A) and a home-built switching box, controlled and monitored by a Labview application. An Ag/AgCl pseudo-reference electrode was connected via a salt bridge to maintain a stable and constant potential over the surface of the MOCSER device. The devices were found to be stable without substantial drifts in the current for more than 24 h in aqueous solutions and about 5 h in simulated gastric fluids (data not shown).

In order to assess the biocompatibility of the polymer-modified surface, human HeLa cells were cultured for 24 h on 4 surfaces (see Figure 1 bottom). The surfaces were bare GaAs, GaAs coated with an MPTMS polymer, GaAs coated with MPTMS/APTMS, and a standard petri dish frequently used for cell-culturing as a control sample. Although the cells did not grow on

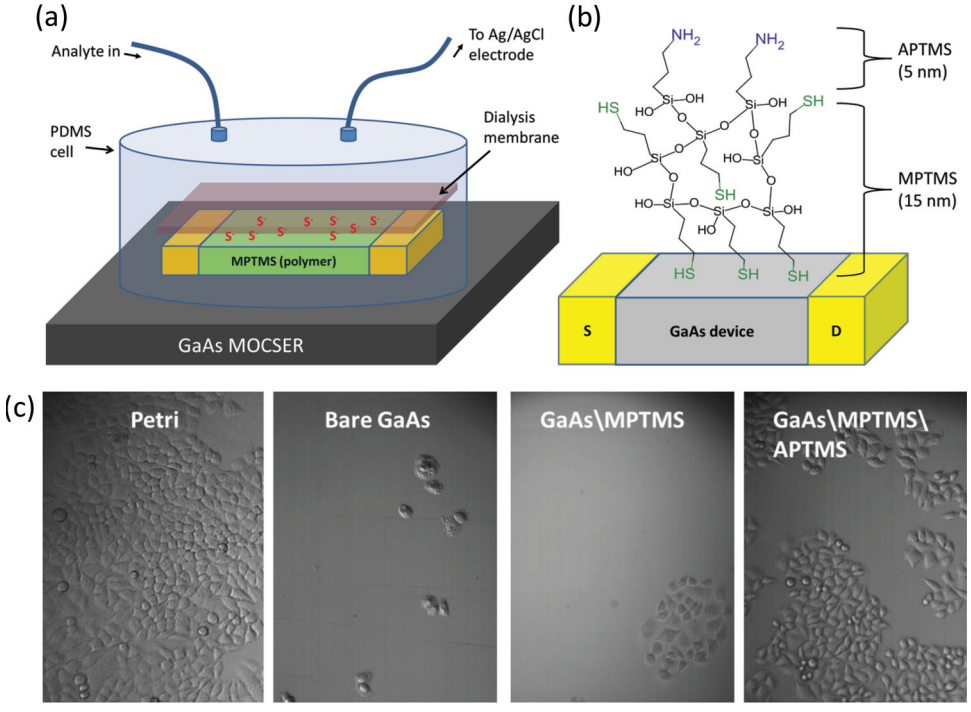

Figure 1. a) Schematic representation of the experimental setup. A syringe pump was used to transfer analyte samples to a MOCSER device, on top of which a PDMS-based microfluidic flow cell was constructed. An Ag/AgCl reference electrode was connected via a salt bridge. The device is coated with polymerized MPTMS and exhibits SH groups, which are partially negative and interact with approaching analytes. A dialysis membrane can be placed on top of the sensing area in order to allow diffusion of only small molecules. b) Scheme of the adsorbed polymers. Two configurations were applied, either with MPTMS alone or MPTMS functionalized with APTMS. c) Biocompatibility of the sensor. Human HeLa cells were cultured for 24 h on 4 substrates: (from left to right) petri dish, bare GaAs, GaAs coated with MPTMS, and GaAs coated with MPTMS/APTMS. Clearly, the coating of the GaAs enables the cells to grow.

bare GaAs, GaAs samples coated with both polymers enabled the cells to grow. Similar results were observed and presented earlier by Kirchner et al.[34]

3. Results

Experiments were conducted in order to verify the mechanism underlying the detection of ammonia by the chemically modified polymer, to establish the sensitivity and selectivity of the sensor, and to confirm its ability to operate in harsh physiological environments. Electrical measurements were carried out by applying a constant potential of 1 V between the source and drain of the MOCSER device (Figure 1) while the current was monitored as a function of time. A constant zero voltage is maintained between the source and the reference electrode during all measurements. Typically, 16 devices set in an array were measured simultaneously. Analytes were introduced and the response of the sensor was measured as the change in source-drain current relative to the base current. The reproducibility between different devices was found to be better than 10%. Since during the measurement all other parameters are kept constant and only the concentration of ammonia is changed, the change in the current upon changing the concentration should be proportional to the concentration of the analyte.

In order to understand the mechanism by which the coated device interacts with ammonia, we monitored the change in the current through the device upon exposure to solutions having various pHs when the polymer is functionalized either with –SH (i.e., MPTMS) or with $–NH_2$ (i.e., APTMS). Experiments were performed in aqueous solutions having various pHs against different added ammonia concentrations. The aqueous solutions were made by titrating water with HCl. Since dissolved ammonia is basic (i.e., $NH_4^+ + OH^-$) when added to aqueous solutions, the pH increases. Hence, the change in the current through MOCSER results from two components: pH variations and the ammonia concentrations. **Figure 2** and **3** present experimental data obtained from the devices when the solutions were exposed to increasing concentrations of ammonia. Figure 2a shows the change in the current through MOCSER coated with MPTMS when ammonia is introduced in the water, whereas Figure 2b shows the behavior of the device when modified with APTMS. In the case of MPTMS, the dependence of this device on the varying concentrations was found to be logarithmic to some extent, as seen in Figure 3a. The minimal concentration that could be detected was about 500 ppb (with a signal-to-noise ratio of 11:1). In the case of APTMS, positive changes in current were recorded for low concentrations of ammonia, whereas negative changes were recorded for concentrations of ammonia above 5 ppm, as seen in Figures 2b and 3b. Figure 3c,d shows the change in the current upon exposure of both devices (MPTMS and APTMS functionalized) to increasing concentrations of ammonia when the solution was set to pH 4. Usually the response of the devices was found to be linear at low concentrations of ammonia, whereas it was logarithmic at higher concentrations, as seen in the insets.

To demonstrate the mechanism by which the sensor operates, devices coated either with APTMS or MPTMS were exposed to the same concentrations of ammonia dissolved in different pH solutions (**Figure 4**). When the device was coated with MPTMS, exposure to 2 ppm of ammonia led to a negative change in currents and the response decreased with acidity until pH 3 (Figure 4a). In the case of APTMS, exposure to 2 ppm of ammonia led to a positive change in currents and the responses decreased with acidity until pH 2 (Figure 4b). At a higher concentration of ammonia, namely 10 ppm, the change in currents for a MPTMS device was negative and decreased with acidity until pH 3, but then became positive at pH 2 and even more so at pH 1 (Figure 4c). Regarding APTMS, the change in current, at the same ammonia concentration, was negative and decreased with increasing acidity, but then became positive at pH 3 and pH 2 (Figure 4d). In all cases, the absolute change in the currents decrease as the solution's pH becomes more acidic, except for the MPTMS-coated device,

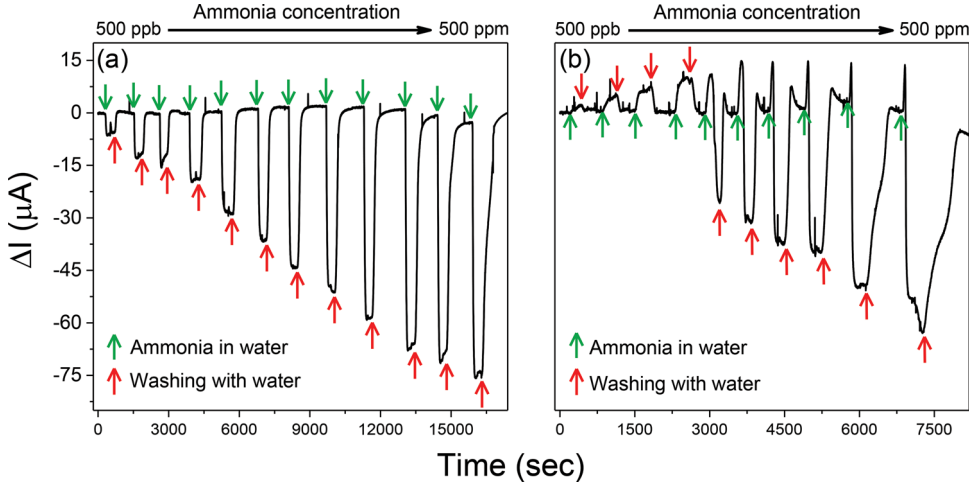

Figure 2. Change in current through a device coated either with MPTMS (a) or APTMS (b) exposed to increasing concentrations of ammonia in water. The stable current for water (without the addition of ammonia) is considered as the baseline and is set to zero. Green arrows indicate the point where ammonia was introduced, whereas red arrows indicate the point of washing.

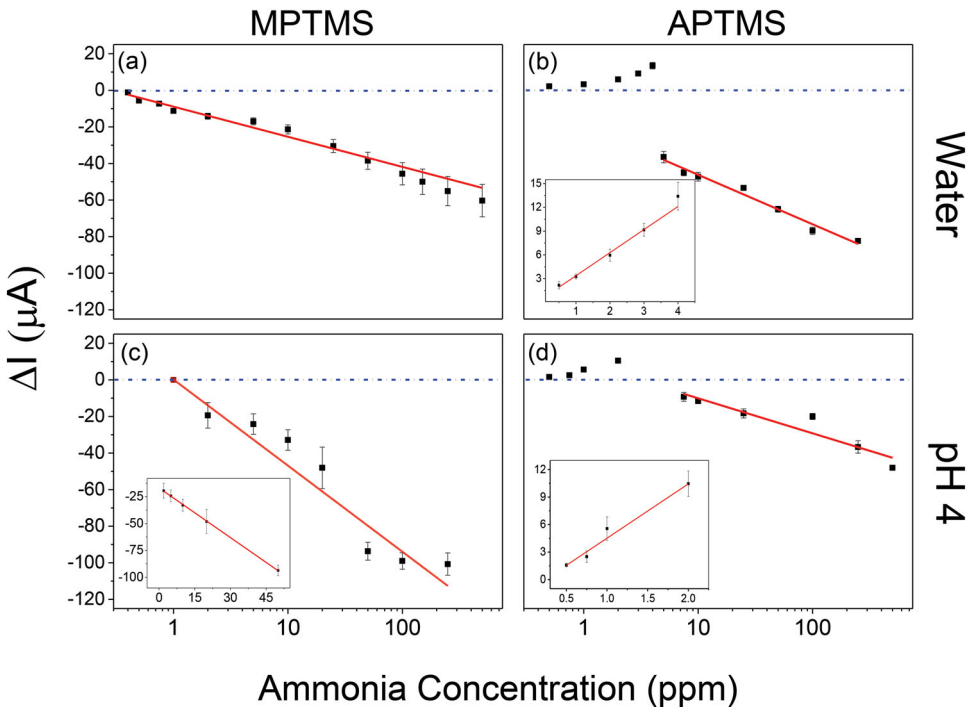

Figure 3. The change in the currents through the MOCSER device when coated with MPTMS and APTMS and exposed to different concentrations of ammonia. The devices were measured in water (a,b) and at pH 4 (c,d). The ammonia concentrations are presented against a logarithmic scale. The inserts show the linear dependence at low ammonia concentrations.

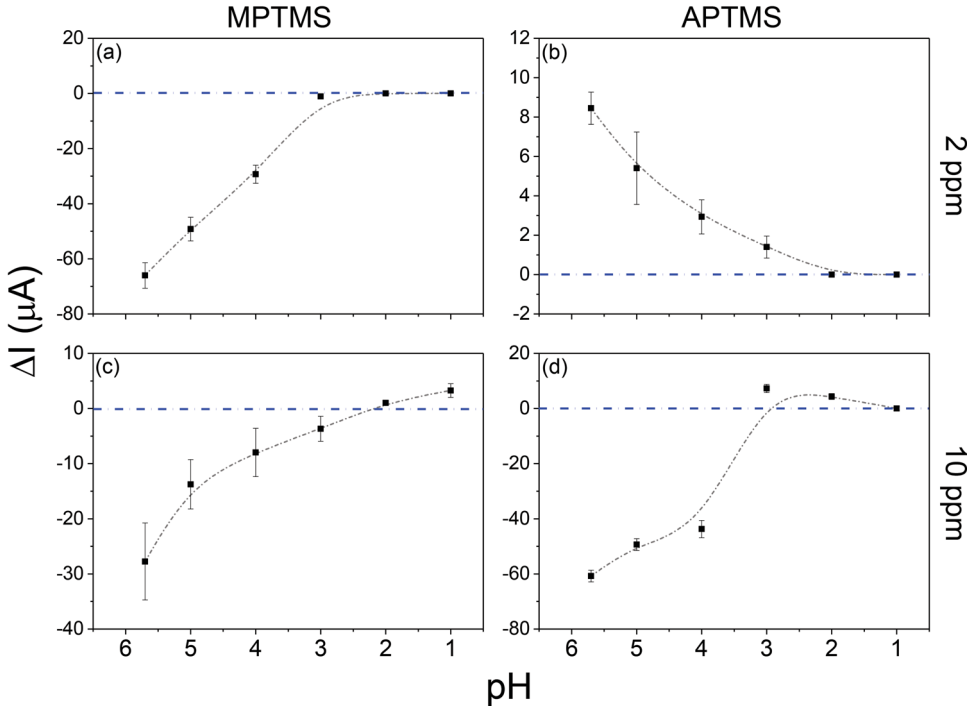

Figure 4. Dependence of the device's responses to constant concentrations of ammonia at varying pH concentrations. Devices coated with MPTMS (a,c) and APTMS (b,d) were exposed to 2 ppm and 10 ppm of ammonia. Positive response were found in (b), (c), and (d) where the effective pH of the analyte is lower than the pI of the surface, whereas they were negative when the effective pH was higher than the pI of the surface (a, c, and d). We noted that the change in currents measured in (a) are higher than those found in (c), although they were expected to be the opposite. This is due to a different device configuration that was applied solely for this set of measurements. Therefore, we consider here only the trends without comparing the absolute values.

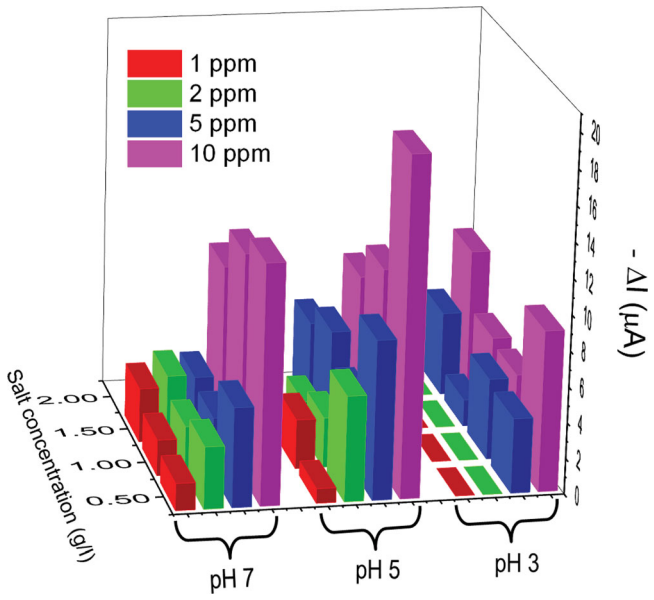

Figure 5. Response measurements for a device coated with MPTMS and exposed to various concentrations of ammonia in different formulations of simulated gastric fluids, namely, for different pH values and different salt concentrations. The response of MOCSER depended more on the pH of the analyte than on the salt concentration.

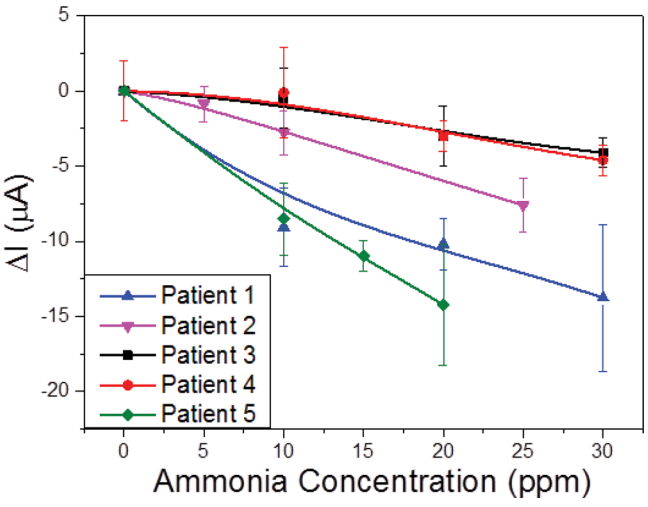

Figure 6. Response measurements for devices coated with MPTMS when different concentrations of ammonia are artificially dissolved in different gastric fluids obtained from actual negative patients. Simulated gastric fluids were used for washing between each measurement. During these measurements the devices were covered with a dialysis membrane. The pH of the gastric fluids was measured before introducing them to the devices and were found to be between pH 1 and 2. The sample from each patient was measured on a different device. The error bars reflects the differences in response observed in the different active channels of the device.

where the response increased when shifting from pH 2 to pH 1.

Although the experiments described above were aimed at establishing the sensing mechanism in a controlled environment, the main challenge is to be able to perform the measurements under realistic physiological conditions. **Figure 5** summarizes the response of the sensor coated with MPTMS to ammonia in different gastric fluid simulations (see also Figure S4, Supporting Information). The parameters that were examined are the salt concentrations and pHs. As observed before, the device is more sensitive at higher pHs; however, it functions well also at lower pH values and at different salt concentrations.

The next test was performed in real gastric fluids taken from different patients observed to be negative for *H. pylori* (**Figure 6**). In this set of experiments, known amounts of ammonia were added to the examined fluids. The sensor was washed with simulated gastric fluids between each measurement. In order to avoid the agglomeration of macromolecules on the surface of the sensor, and thus increase the sensitivity to ammonia in such an environment, a dialysis membrane was placed and fixed on top of the device (see Figure S5, Supporting Information). Clearly, a linear relation was found between the device's response and the increasing concentrations of the added ammonia.

Finally, the sensor was applied for monitoring ammonia in gastric fluids of patients that were found to be negative or positive to *H. pylori*. **Figure 7** shows two typical responses for gastric fluids taken from positive *H. pylori* patients measured against gastric fluids taken from negative *H. pylori* patients. Apparently, responses are being detected by the device with a constant positive change in current. This finding is opposite to the trend measured in Figure 6, where

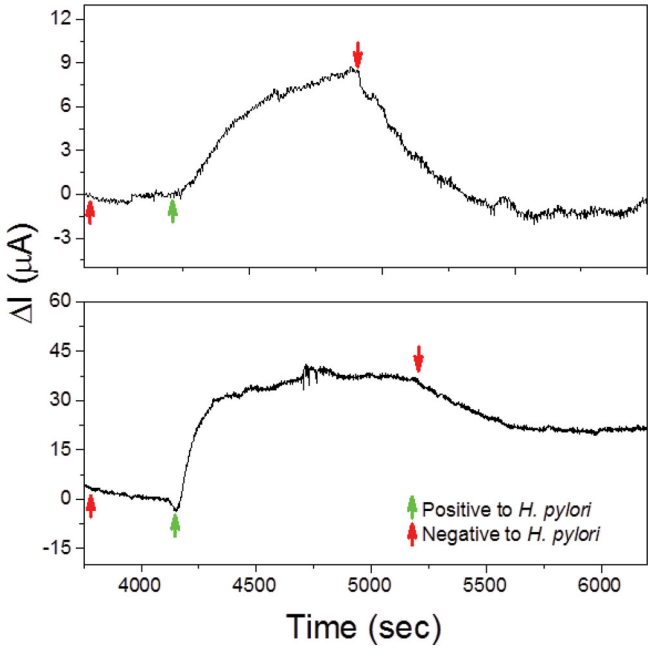

Figure 7. The change in current vs. the time measured for MPTMS-coated devices when exposed to gastric fluids of negative and positive *H. pylori* patients. Gastric fluids of negative patients were used as the baseline. During these measurements the devices were covered with a dialysis membrane. The pH of the gastric fluids was measured before introducing them to the devices and it was found to be between pH 1 and 2. The green arrow indicates the point where a gastric fluid of a positive patient was introduced, whereas red arrows indicate the point where the gastric fluids of a negative patient was used for baseline and washing. The variation in the signal obtained for negative samples is less than 10% relative to the response measured for positive samples (data not shown).

negative patients were introduced to known amounts of ammonia.

4. Discussion

MOCSER is a semiconductor device sensitive to the electrical potential on its surface.[32] Namely, for constant physical parameters, both for the device and system, the change in the channel's source-drain current, ΔI, is determined by the surface/electrolyte interfacial potential (ϕ). The change in interfacial potential results from exposure to the analyte. Utilizing the site binding theory[35] and based on the Nernst equation, the device's responses can be described by the following equation:[36]

$$\Delta I = \alpha \Delta\phi = 2.3\frac{\alpha kT}{q}\left\{\frac{\beta}{(\beta+1)} + \gamma\left[NH_3\right]\right\}(pI - pH) \quad (1)$$

where α is the relation between the source-drain current and the surface potential, q is the surface charge, β and γ are sensitivity parameters, where β is the ratio between the double layer formed by the electrolyte and the thermal potential, and γ reflects the specific affinity to ammonia. For relatively small ranges of ϕ, one can assume that α is constant. The pH is defined for a given solution and pI is the isoelectric point of the polymer coating the surface. When the pH of the solution is higher or lower than the pI of the surface, the surface becomes protonated or deprotonated, respectively. Similarly, the change in surface potential, $\Delta\phi$ (or the change in the current, ΔI), changes its sign from positive to negative when the pH of the analyte, namely, the effective pH, is higher than the pI of the surface. Isoelectric points for the different molecular coatings were calculated from ChemAxon simulations (Marvin sketch) and are listed in **Table 1**. The pI of the MPTMS polymer was found to be about 2.1 whereas the pI of the APTMS-modified polymer is 9.7. To verify the sensing mechanism of ammonia by MOCSER, we monitored the response for ammonia for solutions having different pHs and for two different surfaces with different isoelectric points, namely, MPTMS (-SH) and APTMS (-NH) functionalized surfaces. We measured both the effect of different concentrations of ammonia dissolved in preset environments, namely, water and pH 4 (Figures 2 and 3) as well as fixed concentrations of ammonia in different pH environments (water, pH 5, 4, 3, 2 and 1; Figure 4).

From the results in Figure 2 it is possible to infer the sensing mechanism. Figure 2a shows the change in the current through MOCSER coated with MPTMS when the device is exposed to different concentrations of ammonia in water. The

Table 1. The isoelectric points (pI) of MPTMS and APTMS calculated for single molecules and in their eventual form when used as polymers deposited on the GaAs surfaces. These values were calculated by using simulation from ChemAxon service.

	Single molecule	Polymer
MPTMS	3.3	2.1
APTMS	9.8	10.2
APTMS on MPTMS		9.7

Table 2. The solutions' effective pH measured after addition of various ammonia concentrations (0, 2, 10, and 500 ppm). Measurements were done with a standard pH electrode with an error of ± 0.25.

	0 ppm	2 ppm	10 ppm	500 ppm
Water	5.7	8.8	10.6	11.4
pH 5	5.0	8.8	10.2	11.2
pH 4	4.1	5.2	10.1	11.1
pH 3	3.1	3.1	3.9	11.0
pH 2	2.0	2.2	2.2	10.1
pH 1	1.2	1.3	1.3	1.5

effective pH of the solution, after adding ammonia (**Table 2**), is always higher than the pI of MPTMS (i.e., 2.1). Hence, the response (ΔI) is always negative. For APTMS functionalized devices (Figure 2b), where the pI is 9.7, the effective pH of the solution is lower than the pI at low concentrations of ammonia (from 500 ppb to 5 ppm), and hence ΔI is positive. At higher ammonia concentrations (i.e., greater than 5 ppm) the effective pH of the analyte is higher than the pI of APTMS, and therefore ΔI is negative. Similar behavior was observed when the devices were exposed to different concentrations of ammonia in a solution that was set initially at water and pH 4 (Figure 3).

Figure 4 presents the change in the current through the sensor when a constant concentration of ammonia is introduced to solutions having different pH values. Results are shown for MPTMS and APTMS-coated devices when exposed to 2 and 10 ppm of ammonia. In general, for MPTMS-coated devices, addition of ammonia led to negative changes in current that decreased as the pH of the solution became more acidic (Figures 4a,c). When this device was exposed to 10 ppm of ammonia the responses flipped to a positive change in currents for pH 1 and pH 2 (Figure 4c). Again, this flip occurs just around the point of the pI of MPTMS (i.e., 2.1), as was verified using a calibrated pH electrode (Table 2). Clearly, the addition of ammonia at low pHs did not appreciably change the solution's pH. Hence, the change in the current varies from being negative to positive for pH solutions that are above and below the pI, respectively. This trend is consistent with the model represented by Equation (1).

The case is different, however, for devices functionalized with APTMS, where the pI was calculated to be 9.7. When the device was exposed to 2 ppm of ammonia in solutions having different pHs, the resulting pH of the solution was always found to be lower than the pI (Figure 4b). This is not the case, however, when 10 ppm of ammonia was added to solutions with different pHs. Although the effective pH in solutions ranging from pH 7 to 4 was found to be above the pI value, the pH remained lower than the pI value for more acidic solutions (Figure 4d and Table 2). Apparently for this coating, the change in the current follows the sign of the resulting $(pI - pH)$ term in Equation (1), whereas its value does not vary as predicted by this equation. This observation probably results from the complexity and the porosity of this coating, where the underlying layer of MPTMS also plays a role in the sensing process. As APTMS is more hydrophilic than MPTMS, the hydration layer changes, resulting in a change of the dielectric properties of the layer, and in turn, the surface potential ($\Delta\phi$).[37]

To verify the sensing capabilities of ammonia in real physiological environments, we measured primarily the responses of the sensors in controlled simulated gastric fluids. The salt concentrations (NaCl) and the pH were varied in order to record their influence on the device's sensitivity against different ammonia concentrations (Figure 5). In general, it was found that although the pH has a strong effect on the sensitivity, the salt effect is relatively small (see also Figure S4, Supporting Information). The MOCSER is not simply a pH sensor, since we observed a clear response when ammonia is added to pH 1 gastric simulation (Figure S3, Supporting Information) while the pH of the solution is not changed upon addition of ammonia.

However, when real gastric fluids were obtained from patients, these fluids had large quantities of undissolved and precipitated carbohydrates, agglomerated fats, and denatured proteins. These macromolecules block the MOCSER sensing area and screen the sensitivities to ammonia (see Figure S5a, Supporting Information). When the fluid is filtered, prior to introducing the device, we could obtain detectable responses (Figure S5b, Supporting Information) with sensitivity down to about 20 ppm of ammonia (data not shown). However, external filtration of gastric fluids collected from the patients is time consuming. This can be avoided by placing a filtering membrane on top of the sensing area. For this purpose, we used a 10 kDa semi-permeable dialysis membrane that was placed directly on top of the MOCSER sensing area (see Figure 1).

Figure 6 presents the response from unfiltered gastric fluids obtained from 5 different patients. In this set of experiments the standard gastric simulation was used for washing. All responses were normalized by measuring the difference between the spiked and the unspiked gastric fluids. The sensitivity, or the limit of detection (LOD), obtained with this configuration was better than 10 ppm of ammonia, when using the dialysis membrane, with a signal-to-noise ratio of 5:1. The limit of quantification (LOQ) was found to be 20 ppm. The error-bars in Figure 6 reflect the variability in the responses obtained from these gastric fluids. It is important to realize that gastric fluids taken from positive-infected *H. pylori* patients are known to have at least 100 ppm of ammonia; hence, the sensitivity we obtained here exceeds the requirement needed for detection of *H. pylori*. This is verified in Figure 7 where the results are shown for samples taken from a patient diagnosed as positive to *H. pylori*. Here, a clear difference in the response of the device was observed between these two samples. However, since the measured responses are opposite to the consistent trends presented in Figure 6, where negative patients were introduced to known amounts of ammonia, we can conclude that a gastric fluid extracted from a positive patient is not equal to that of a negative patient plus ammonia.

5. Conclusions

The ability to apply semiconductor devices for sensing metabolites in biological environments opens up the possibility of taking advantage of the microelectronic-based technologies in real-time applications. In the present work we propose a novel mechanism of detecting ammonia species by using a nanometric polymer coating over a molecular electronic transducer. The polymer serves both as a protective layer against surface oxidations, and as an electrostatic receptor layer. We demonstrate the response of the polymer in varying acidic environments and its sensitivity to minute amounts of ammonia. We further demonstrate that ammonia can be sensed even in real gastric fluids with high sensitivities. The proposed devices can function for hours within physiological solutions without becoming damaged or intoxicating the environment. By introducing different surface functionalities, an array of sensors can be constructed, thus reducing the possibility of false-positive responses and allowing detection of ammonia without the need to pre-calibrate individual devices.

Since the sensor developed combines sensitivity and selectivity with short measuring times and low production costs, it is an attractive means for continuous sensing of ammonia both under in-vivo and ex-vivo conditions.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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[1] L. R. Meyerson, K. D. McMurtrey, V. E. Davis, *Anal. Biochem.* **1978**, *86*, 287.
[2] Y. M. Fraticelli, M. Meyerhoff, *Anal. Chem.* **1981**, *53*, 1857.
[3] E. Bekyarova, M. Davis, T. Burch, M. Itkis, B. Zhao, S. Sunshine, R. Haddon, *J. Phys. Chem. B* **2004**, *108*, 19717.
[4] S. Alegret, J. Alonso, J. Bartroli, M. Del Valle, *Anal. Chim. Acta* **1990**, *231*, 53.
[5] M. Meyerhoff, R. Robins, *Anal. Chem.* **1980**, *52*, 2388.
[6] A. P. F. Turner, *Sens. Actuators* **1989**, *17*, 433.
[7] R. Lal, *Bioelectrochem. Bioenerg.* **1992**, *27*, 121.
[8] N. Jaffrezic-Renault, K. Wan, A. Senillou, J. Chovelon, C. Martelet, *Analusis* **1999**, *27*, 578.
[9] A. Senillou, N. Jaffrezic-Renault, C. Martelet, S. Cosnier, *Talanta* **1999**, *50*, 219.
[10] I. Humenyuk, B. Torbiero, B. S. Assie-Souleille, R. Colin, X. Dollat, B. Franc, P. Temple-Boyer, *Microelectron. J.* **2006**, *37*, 475.
[11] H. Liu, J. Kameoka, D. Czaplewski, H. Craighead, *Nano Lett.* **2004**, *4*, 671.
[12] P. Bergveld, *Biosensors* **1986**, *2*, 15.
[13] K. Najafi, *Sens. Actuators, B* **1990**, *1*, 453.
[14] K. Tsukade, M. Sebata, Y. Miyahara, H. Miyagi, *Sens. Actuators* **1989**, *18*, 329.
[15] E. Autret-Lecaa, A. P. Jonville-Béraa, M. Llaua, F. Bavoux, J. Saudubray, J. Laugier, P. Barbier, *J. Chronic Dis.* **2001**, *54*, 857.
[16] H. Miyaji, S. Ito, T. Azuma, Y. Ito, Y. Yamazaki, Y. Ohtaki, F. Sato, M. Hirai, M. Kuriyama, M. Kohli, *Gut* **1997**, *40*, 726.

[17] N. Uemura, S. Okamoto, S. Yamamoto, N. Matsumura, S. Yamaguchi, M. Yamakido, K. Taniyama, N. Sasaki, R. J. Schlemper, *N. Engl. J. Med.* **2001**, *345*, 784.
[18] The Eurogast Study Group, *J.-Lancet.* **1993**, *341*, 1359.
[19] D. J. E. Cullen, G. M. Hawkey, D. C. Greenwood, H. Humphreys, V. Shepherd, R. Logan, C. J. Hawkey, *Gut* **1997**, *41*, 459.
[20] R. Chittajallu, W. Neithercut, A. Macdonald, K. McColl, *Gut* **1991**, *32*, 21.
[21] F. Megraud, E. Neman-Simha, D. Brugmann, *Infect. Immun.* **1992**, *60*, 1858.
[22] J. E. Domínguez-Muñoz, A. Leodolter, T. Sauerbruch, P. Malfertheiner, *Gut* **1997**, *40*, 459.
[23] Thermo Scientific - Orion standard ammonia ion selective electrode (n.d.), http://www.instrumart.com/assets/ISEammonia_manual.pdf (accessed: April 2013).
[24] A. Radomska, R. Koncki, K. Pyrzynska, S. Glab, *Anal. Chim. Acta* **2004**, *523*, 193.
[25] E. Mohabbati-Kalejahi, V. Azimirad, M. Bahrami, A. Ganbari, *Talanta* **2012**, *97*, 1.
[26] A. Salimi, R. Hallaj, G. R. Khayatian, *Electroanalysis* **2005**, *17*, 873.
[27] J. A. Killard, R. M. Smyth, *Trends Biotechnol.* **2000**, *8*, 433.
[28] R. M. Vilar, J. El-Beghdadi, F. Debontridder, R. Naaman, A. Arbel, A. Ferraria, A. Botelho Do Regod, *J. Mater. Sci. Eng. C* **2006**, *26*, 253.
[29] E. Capua, R. Cao, C. N. Sukenik, R. Naaman, *Sens. Actuators, B* **2009**, *140*, 122.
[30] A. K. Tatikonda, M. Tkachev, R. Naaman, *Biosens. Bioelectron.* **2013**, *45*, 201.
[31] D. Bavli, M. Tkachev, H. Piwonski, E. Capua, I. Albuquerque, D. Bensimon, R. Naaman, *Langmuir* **2010**, *28*, 1020.
[32] E. Capua, A. Natan, L. Kronik, R. Naaman, *ACS Appl. Mater. Interfaces* **2009**, *1*, 2679.
[33] M. Tkachev, A. K. Tatikonda, A. Bitler, R. Guliamov, R. Naaman, *Engineering* **2013**, *5*, 1.
[34] C. Kirchner, M. George, B. Stein, W. J. Parak, H. E. Gaub, M. Seitz, *Adv. Funct. Mater.* **2002**, *12*, 266.
[35] D. E. Yates, S. Levine, T. W. Healy, *J. Chem. Soc., Faraday Trans. 1* **1974**, *70*, 1807.
[36] L. Bousse, N. F. Rooij, P. Bergveld, *IEEE Trans. Electron Devices* **1983**, *ED-30*, 1263.
[37] R. Sandifer, *J. Anal. Chem.* **1988**, *60*, 1553.