Development of chemical field effect transistors for the
detection of urea

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Abstract

Low cost, disposable, chemical field effect transistor (ChemFET) microsensors including a SiO$_2$/Si$_3$N$_4$ pH-sensitive gate, a ChemFET/ReFET structure and a titanium/gold pseudo-electrode have been fabricated using a standard P-well silicon technology. The fabrication process is described and sensor properties and performances are demonstrated through pH measurements. The pH-ChemFET is adapted to biochemical applications owing to photosensitive polyvinyl alcohol (PVA) layers patterned by standard photolithography techniques. Application is performed through the development of urease-based enzymatic field effect transistor (U-EnFET) for the detection of urea. The microsensor will be used for blood analysis and more precisely for hemodialysis.

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1. Introduction

The blood analysis techniques require the development of smart biochemical microsensors with the following characteristics: CMOS compatibility, mass fabrication, low cost, low power and ease of use. Chemical field effect transistors (ChemFETs) provide all these advantages and their use has been demonstrated through the development of enzymatic and biochemical field effect transistor (EnFET and BioFET) for many applications [1–4]. However, up to now, their industrial development is still balanced by several drawbacks related to their interface with liquid solutions (manufacturing of an integrated reference electrode, electrical insulation, packaging, temporal drift, etc.) and to the CMOS compatibility of the different (bio)chemical sensitive materials (individual fabrication process, etc.). Nevertheless, since the low cost requirement will be always of first priority for industrial applications, these drawbacks must be got round while developing collective technological processes for the mass fabrication of ChemFETs and BioFETs sensors [5–7].

This paper deals with the development of such standard, CMOS compatible, technological process for the manufacturing of low cost pH-sensitive field effect transistors (pH-ISFET) and urea-sensitive enzymatic field effect transistors (U-EnFETs).

2. Experimental

Ion sensitive field effect transistors (ISFETs) were fabricated on (110)-oriented N-type (500 Ω-cm) silicon substrate. The insulation between the electrical active zones and the electrolyte was performed using a standard P-well technology, leading to the fabrication of N-channel ChemFETs (Fig. 1). A 50 nm thermally grown SiO$_2$ layer and a 50 nm Si$_3$N$_4$ layer deposited on top formed the pH-sensitive gate structure.

Then, the sensor applications have been extended to enzymatic detection, and more especially for urea detection, by using biocompatible, hydrophilic and photosensitive polyvinyl alcohol (PVA) layers [8]. After diluting urease in an aqueous PVA solution, spin coating and UV photolithography techniques have been developed and optimised in order to pattern urease-based PVA thin films (thickness lower than 10 μm). Thus, enzymatic layers have been collectively deposited on top of the SiO$_2$/Si$_3$N$_4$ pH-sensitive gate in order to form a SiO$_2$/Si$_3$N$_4$/PVA urea-sensitive gate (Fig. 2).

Finally, the chemical sensors were manufactured on a 5 mm × 5 mm chip. It encloses a ChemFET/ReFET structure to improve detection properties by differential analysis [9,10], and a titanium/gold (200/800 nm) pseudo-electrode to bias the electrolyte/insulator/semiconductor (EIS) gate structure (Figs. 2 and 3). The chips were stuck owing to an epoxy insulating glue on specifically coated printed circuit. After wire bonding, encapsulation was finally performed.
using a biocompatible silicone and leaving the sensitive parts uncovered (Fig. 3).

The gate voltage being applied to the solution either by a reference calomel electrode, either by the titanium/gold pseudo-electrode, chemical sensors were characterised by $I-V$ measurements, using a HP4140B picoparameter. Drain-source current $I_{DS}$ was measured while gate-source voltage $V_{GS}$ underwent variations, the P-well being connected to the source and the substrate being not connected. Chemical detection properties were finally determined by studying the potential shift of the $I-V$ curves.

pH measurements were studied using three standard buffer solutions (pH $= 4.01$, 7.00 and 10.01). Enzymatic detection was performed by diluting urea (purchased from Sigma) in citrate-phosphate buffered (pH $\approx 7$) solutions or in dialysate (pH $\approx 7.6$), for concentrations ranging between 5 and 50 mmol/l. All the measurements were done at a constant temperature (20$^\circ$C).

3. pH detection

The performances of the SiO$_2$/Si$_3$N$_4$ ISFET chemical sensor have been studied thanks to the H$^+$ ion detection (Fig. 4). A quasi-Nernstian pH response (pH sensitivity around 55 mV/pH) has been evidenced while using the calomel reference electrode. When the titanium/gold pseudo-electrode is used, a linear pH response is still evidenced but the pH sensitivity is decreased towards 40 mV/pH (detection yield of 66% compared to the Nernst law). This result demonstrates that the titanium/gold metallic electrode can be used as gate electrode without major drawbacks [5,9].

Finally, since the ISFET and ReFET sensors are characterised by similar pH responses whatever the electrode chosen, the differential analysis shows no pH detection properties (pH sensitivity lower than 0.01 mV/pH). This result demonstrates the good use of the ChemFET/ReFET structure.
4. Urea detection

The SiO$_2$/Si$_3$N$_4$/PVA(urease) structure has been tested as sensitive gate for the development of U-EnFET. Its detection principle is based on the production of acid or/and basic chemical species due to the urease enzymatic reaction, i.e. due to the urea CO(NH$_2$)$_2$ hydrolysis:

$$\text{CO(NH}_2\text{)}_2 + 2\text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3 + \text{H}_2\text{CO}_3$$

(1)

The production of the ammonia base NH$_3$ and of the carbonic acid H$_2$CO$_3$ in aqueous solution is responsible for chemical reactions between the following acid/base couples: NH$_4^+$/NH$_3$, H$_2$CO$_3$/HCO$_3^-$, HCO$_3^-$/HCO$_2^2$-, H$_3$O$^+$/$\text{H}_2$O and $\text{H}_2$O/OH$^-$:

$$\text{NH}_4^+ + \text{H}_2\text{O} \leftrightarrow \text{NH}_3 + \text{H}_3\text{O}^+$$

(2)

$$\text{H}_2\text{CO}_3 + \text{H}_2\text{O} \leftrightarrow \text{HCO}_3^- + \text{H}_3\text{O}^+$$

(3)

$$\text{HCO}_3^- + \text{H}_2\text{O} \leftrightarrow \text{CO}_3^{2-} + \text{H}_3\text{O}^+$$

(4)

$$2\text{H}_2\text{O} \leftrightarrow \text{H}_3\text{O}^+ + \text{OH}^-$$

(5)

Since the dissociation constants of these different acid/base couples are well known [11], the chemical system given by Eqs. (1)-(5) can be easily studied provided that the urease enzymatic reactions is defined. For our calculations, the urease activity $a$ has been kept constant and the urea concentration has been assumed to be infinite. Thus, in a given volume $V$, the concentrations of produced ammonia [NH$_3$]$_p$ and carbonic acid [H$_2$CO$_3$]$_p$ as a function of the time $t$ is given by:

$$[\text{NH}_3]_p = 2[H_2\text{CO}_3]_p = \frac{at}{V}$$

(6)

Of course, Eq. (6) is not realistic but it allows to study globally the influence of the urease activity on the pH variations.

Fig. 5 represents such variations as a function of time and for different initial conditions pH$_0$. Whatever the pH$_0$ value, the solution pH is found to tend towards a constant value pH$_\infty$ around 9.17. Therefore, the urea hydrolysis is found to be responsible for a pH increase or decrease whether pH$_0$ is lower or higher than pH$_\infty$. In the proposed U-EnFET sensor, these pH variations occur locally in the PVA enzymatic film and are finally detected by the SiO$_2$/Si$_3$N$_4$ pH-sensitive structure.

The first studies have been done in citrate-phosphate buffered solutions (pH $\approx$ 7) with various urea concentrations (Fig. 6). The U-EnFET detection properties are characterised on a wide concentration range, evidencing a pH increase, in agreement with the previous calculations as well as results from literature [2,10,12,13]. However, they must be balanced since the pH-ReFET is also undergoing variations in the same range. Since buffered solutions have been used to prevent them, these variations are not easy to understand. Different explanations can be proposed. First, it can be assumed that, since the urease activity is globally responsible for a constant consumption of H$^+$ ions in the PVA film (Eqs. (1)-(4)), a pH increase is confined near the SiO$_2$/Si$_3$N$_4$/PVA gate structure. This increase should be responsible for a pH gradient, i.e. for H$^+$ ion diffusion phenomena, in the U-EnFET proximity. Finally, these diffusion phenomena could be detected by the pH-ReFET. Second, since the measurements have been done from the lowest to the highest urea concentrations, the voltage variations could be related to the ChemFET temporal drift. All in all, both effects could interfere simultaneously and be responsible for the pH-ReFET variations evidenced in Fig. 6.

Nevertheless, this drawback can be tackled off by using a differential analysis between the U-EnFET and the pH-ReFET. Thus, a quasi-linear response is finally
evidenced for urea concentrations ranging from 5 to 50 mmol/l and the urea detection sensitivity has been estimated around 1 mV/mmol/l, i.e. around 45 mV/pUrea.

To go further, the sensor detection properties have been tested in biological solutions obtained by mixing urea with various concentrations into dialysate (pH ≈ 7.6) (Fig. 7). Compared to results obtained previously in buffered solutions, both U-EnFET and pH-ReFET show higher voltage variations in the [5–50 mmol/l] urea concentration range. This phenomenon demonstrates if necessary that buffered solutions prevent the pH from increasing in the proximity of the SiO2/Si3N4/PVA(urease) gate structure.

It appears that the U-EnFET detection properties are saturated for urea concentrations higher than 25 mmol/l. This saturation effect can be explained by considering reaction kinetics. For high enzyme concentrations, the reaction rate is controlled by the enzyme characteristics. In our case, for high urea concentration, detection properties are therefore limited by the enzymatic properties of the urease-based PVA layer: concentration, activity, etc.

As previously discussed, the comparison between the U-EnFET and the pH-ReFET must be undergone. Since the two sensors show similar response and saturation effects, the pH-ReFET variations could be related to an affective solution pH increase due to the urea hydrolysis and/or to H+ ion diffusion phenomena in the U-EnFET proximity but no longer to a temporal drift (see below).

Finally, the differential analysis between the U-EnFET and the pH-ReFET evidences the urea detection for concentrations ranging from 5 to 25 mmol/l.
5. Conclusions

A CMOS compatible, low cost, disposable, SiO$_2$/Si$_3$N$_4$ pH-chemical microsensor including a ChemFET/ReFET structure and a titanium/gold pseudo-electrode has been fabricated. The pH detection properties have been studied, evidencing a 66% detection yield (compared to the Nernst law) while using a titanium/gold gate electrode, and demonstrating the good use of the ChemFET/ReFET structure for differential analysis.

Spin coating and UV photolithography mass-fabrication techniques have been developed in order to deposit and pattern PVA enzymatic films. Thus, the ChemFET sensor has been adapted to urea detection and U-EnFETs have been fabricated. Finally, the U-EnFET and pH-ReFET detection properties have been studied and compared for the urea detection in buffered solutions and dialysate, characterising detection range, saturation phenomena and/or detection sensitivity.

These works will be continued by studying the influences of the PVA films characteristics (geometry, thickness, etc.) and the urease characteristics (concentration, activity, etc.) in order to improve the urea detection properties for future applications in blood analysis and more precisely for hemodialysis.

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References


Biographies

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