An Extended Gate Field Effect Transistor-based (EGFET-based) Urea Microbiosensor Based on Polypyrrole

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***Abstract*—Here, an extended – gate field effect transistor (EGFET) urea microsensor based on modified polypyrrole (PPy) is reported for the quantitative detection of urea in aqueous solution. the EGFET urea sensor was made by integrating a small and cheap metal oxide semiconductor field - effect transistor (MOSFET) with a Au microelectrode modified with urease and pH sensitive PPy. First, the urease was added to a pyrrole solution and then pyrrole/urease solution was electropolymerized on the surface of gold microelectrode in galvostanic mode to produce a urea sensitive microelectrode. The microsensor was imaged using a stereo microscope to confirm the polymerization of pyrrole/urease. The EGFET urea microsensor was tested in deionized water containing various concentrations of urea. The electrode showed a linear response for a wide concentration range of urea from 10^{-9} to 10^{-5} M with a sensitivity of 35.5 mV/decade urea.**

***Keywords*—EGFET, urea, urease, polypyrrole, microsensor, electropolymerization**

I. Introduction

Urea (CH_4N_2O) is a nitrogenous organic compound that is one of the metabolic products of protein metabolism. Metabolism of proteins leads to the production of ammonia which then can be converted into urea in kidney and liver. Proper control of urea is essential for environmental monitoring, farming science, food science and medical diagnosis. Urea level is good marker for renal functioning, dehydration, increased catabolism of proteins, high protein diet, malnutrition, pregnancy, inhibition in urinary system, shock and stress [1]. Therefore, accurate and sensitive measurement of urea level is very crucial for medical diagnosis. Amperometric, thermal, colourimetric, chromatographic, conductometric and potentiometric methods are available for urea sensing applications [1][2]. In the biosensor field, urea biosensors are frequently based on pH-sensing electrodes [3]. Polymers are widely used in the field of biosensors due to their easy depositing with electrochemical methods, control of coating thickness, redox conductivity of the polymer and polyelectrolyte properties. In a study, Rajesh et al. designed a potentiometric urea biosensor by covalently immobilizing urease on a conducting copolymer poly(N-3-aminopropyl pyrrole-co-pyrrole) (PAPCP) film. Polymer was electrochemically prepared onto an indium–tin-oxide (ITO) coated glass plate. High enzyme loading and increased stability of the enzyme electrode were achieved by the covalent linkage of the enzyme and porous morphology of the polymer film. Moreover, the electrode demonstrated a linear response in the range of 6.3 x 10^{-6} to 4.07 x 10^{-4} M urea with a sensitivity of 27.5 mV per decade [4]. Polypyrrole (PPy) has high chemical stability, easy oxidation and low monomer cost [5]. Similarly, a sensitive and stable urea biosensor can be made by covalently immobilizing the urease enzyme to the pH sensitive PPy polymer or by entrapping the enzyme through the electrodeposition of the PPy film onto the electrode [6]. In this context, Komaba et al. developed an urea sensor by entrapping urease enzyme in PPy onto a Pt electrode through electropolimerization in potentiostatic mode. A sensitivity of 31.8 mV/decade urea was obtained with a linear response in the range of 1 x 10^{-4} to 3 x 10^{-1} $mol.dm^{-3}$ [7].

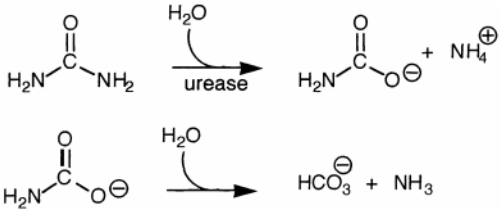

Fig. 1: The chemical equation for catalysis of urea by urease[8]

In most enzyme-based biosensors, various binders are used to keep the enzymes on the electrode surface. In a different study, bovine serum albumin (BSA) was used to bind urease enzyme onto an indium–tin-oxide (ITO) coated glass plate. In this study, a potentiometric urea biosensor was produced by electropolymerization of PPy onto an ITO coated glass plate. The sensitivity of the sensor was 17.3 mV/decade urea and it showed a linear response in the range of 6.6 × 10^{-6} to 7.5 × 10^{-4} M urea [9].

In this study, a new extended–gate field effect transistor-

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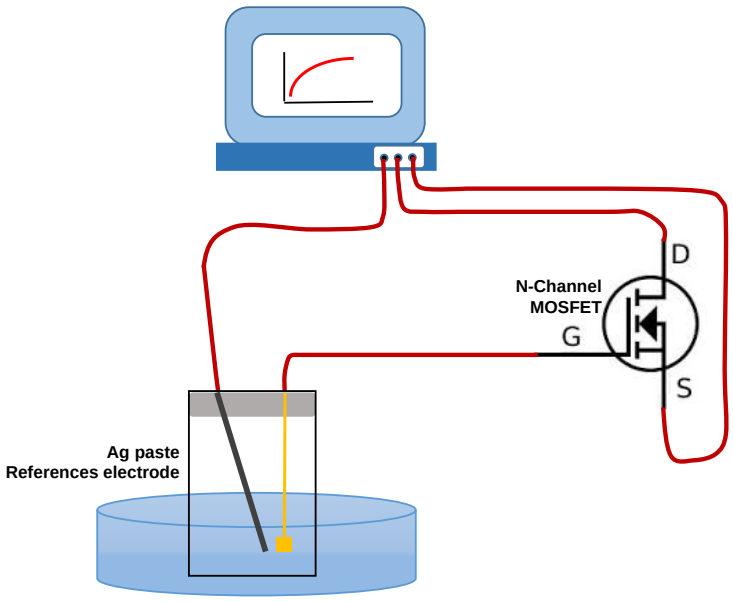

Fig. 2: EGFET pH sensor constructed through the integration of a PPy coated electrode and a MOSFET.

based (EGFET) urea microbiosensor was developed through the entrapment of urease in PPy matrix on a gold microelectrode. Although entrapment of urease in PPy through electropolimerization is not a new approach, using the approach for the fabrication of an EGFET-based urea microbiosensor is new to the best of our knowledge. According to the results, the microbiosensor successfully measured urea with good sensitivity and reliability.

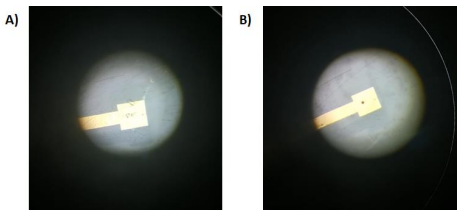

Fig. 3: Image of gold microelectrode (A) before and (B) after electropolymerization of pyrrole on chip.

II. MATERIAL AND METHODS

A. Material

Phosphate buffered saline (PBS) (Sigma Aldrich, USA), pyrrole (Sigma-Aldrich, USA), lithium perchlorate ($LiClO_4$) (Sigma-Aldrich, USA), perchloric acid ($HClO_4$) (Supelco, USA), silver paste (Sigma-Aldrich, USA), n-type MOSFET (Metal Oxide Semiconductor Field Effect Transistor) (IRFZ44N, International Rectifier, USA), urease from canavalia ensiformis (Jack bean) (Sigma-Aldrich, USA), urea (Sigma-Aldrich, USA). Potentiostat (Autolab PGSTAT204) was used in the electropolymerization of pyrrole/urease on the produced sensor. The field effect transistor (FET) analysis unit (B2901A Precision Source/Measure Unit) was used for electrochemical measurements and characterization of the produced sensor.

B. Microchip production

A glass slide was spin coated with S1818 positive photoresist at 2000 rpm for 30 sec. After prebaking, the photoresist was patterned via contact lithography. The exposed areas of the photoresist was removed using a developer solution (CD-26, Microchem,USA). Titanium (Ti) and gold (Au) films were sputtered on the glass slide with patterned photoresist. Lastly, the remaining photoresist was removed using acetone to define the Au patterns of the microchip [10], [11], [12].

C. Pyrrole/urease solution preparation and electropolymerization

A pyrrole solution was prepared by using 0.2 M pyrrole, 0.2 M lithium perchlorate and 0.1 M perchloric acid. 4 mg of urease was dissolved in 75 µl of pyrrole solution. Electropolymerization of the pyrrole-urease solution was carried out in galvanostatic mode by applying a current of 0.1 µA for 180 seconds [13]. A gold microelectrode and an Ag electrode printed on the chip was used as working electrode and reference electrodes, respectively. The electrode coated with PPy/urease was incubated at room temperature for 40 min. 10^{-9} mol/L, 10^{-8} mol/L, 10^{-7} mol/L, 10^{-6} mol/L and 10^{-5} mol/L urea solutions were used for testing the analytical performance of the EGFET-based urea microbiosensor.

D. Use and characterization of EGFET urea microsensor in urea measurement after integration with MOSFET

The analytical performance of the EGFET-based urea microbiosensor was assessed using a FET analyzer unit in solutions with different concentrations of urea. While the source and drain of the n-type MOSFET were connected to the source and drain inputs of the FET analyzer unit, the gate is connected to the EGFET-based urea microbiosensor [14], [15].

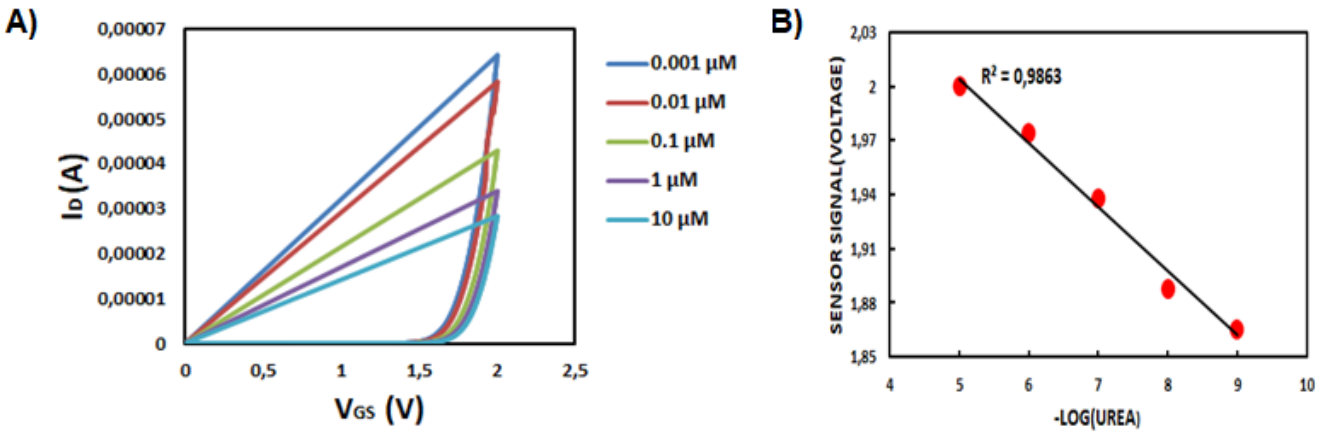

Fig. 4: I-V curves obtained at different urea concentration (A) Obtained using I_D values measured at V_{GS} (0 - 2 V) for each urea concentration (B) Calibration curve .

Measurements were carried out by immersing the EGFET-based urea microbiosensor into the urea test solutions. The potential applied to the gate was scanned over a wide range (0 to +2 V), while a constant potential was applied between the source and the drain (+0.5 V). Thus, current-voltage curves (i-v) were obtained.

III. RESULT

Electropolymerization of the pyrrole-urease solution was achieved in galvanostatic mode with a constant current of 0.1 µA. The coated microsensor was incubated at room temperature for 40 min to improve the stability of the sensor. The electropolymerization process was carried out using a two-electrode system. The reference electrode was printed on the chip with Ag paste so as to keep the distance between the working (gold electrode) and the reference electrode constant throughout the study. After the PPy-urease electropolymerization, it was observed that the color of the Au electrode changed to black as can be seen in Figure 4. The color change confirms that the electropolymerization of the PPy-urease was successful.

The urea sensitive microsensor was integrated with an n-type MOSFET as shown in Figure 2. The EGFET-based urea microbiosensor was immersed in solutions with varying concentrations to obtain I_D-V_{GS} curves. Basically, the urease enzyme in the PPy catalyzes the hydrolysis of urea and produces ammonia, which then lowers the pH near the surface of the EGFET-based urea microbiosensor. As can be seen in Figure 3, the obtained I_D-V_{GS} curves differed depending on the urea concentration. The V_{GS} of the MOSFET is modulated by the sensing layer depending on the urea concentration. In other words, different concentrations of urea solutions changed the conductivity of the PPy, affecting the potential applied to the channel between source and drain in the MOSFET. Hereby, different I_D-V_{GS} curves were obtained at different urea concentration values. The calibration curve based on V_{GS} obtained from I_D-V_{GS} graph with respect to different concentrations of urea showed that the relationship between the urea concentration and the V_{GS} was linear (R^2 = 0.9863) and the sensitivity was 35.5 mV/decade urea. The EGFET-based urea microsensors may be suitable for various portable applications, including point of care testings.

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