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# Urea Biosensor Using $NH_3$ Nitrided Amine Groups on Flexible Substrate

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**Abstract**

In this study, indium tin oxide (ITO) layers were deposited on polyethylene terephthalate (PET) substrates (ITO/PET) as a sensing membrane. In order to generate the amine groups on the surface for urease immobilization, $NH_3$ plasma was used with RF power of 100 W for various times. The pH sensitivities of ITO/PET electrodes treated without and with $NH_3$ plasma for 3 and 6 min were all around 51±4 mV/pH. However, the pH sensitivities of the samples treated with $NH_3$ plasma for 9 min were slightly reduce to 45±5 mV/pH. In addition, the urea sensitivities of ITO/PET EGFET can be increased from 20.7 to 43.3 mV/p$C_{urea}$ by $NH_3$ plasma from 3 to 9 min.

**Key words:** indium tin oxide, polyethylene terephthalate, amine groups, $NH_3$ plasma, urea

## Introduction

Extended gate field effect transistors (EGFET) were developed based on ion sensitive field effect. The sensor electrode is extended from a conventional MOSFET, and keeps the FET device away from the measurement environment [1]. In addition, the sensor electrodes are easy fabricated and could be disposable. Till now, many materials, such as $SnO_2$ [1], $TiO_2$, ZnO [2], ITO [3], have been applied on the sensing membrane of EGFET. Considering to the biocompatibility and cost down, the sensing electrodes were also fabricated on the flexible substrate, such as polyethylene terephthalate (PET).

Urea biosensors with urease on sensing membrane were immobilized with many enzyme immobilized methods, such as physical adsorption, entrapment , covalent bonding [4], cross-linking**.** The $H^+$ or $NH_4^+$ ions which are the products of urea hydrolysis could be detected by urea biosensor. Due to the time-consuming of covalent bonding process, more methods were published to simplify the process by coating polymer membrane or increasing the active area to enhance the efficiency of bonding. In the way of cross-linking process, the reaction between amine bond and aldehyde on glutaldehyde (GA) plays an important role for pUrea-sensing. Therefore, creation of amine bond on sensing membrane surface by inorganic method seems a possible way to fabricate the urea sensor. In this study, $NH_3$ plasma treatment with RF power of 100W for various time was proposed to create the amine groups on ITO sensing membrane to replace the covalent bonding procedures.

## Experimental

For the ITO/PET electrode, ITO layers deposited on polyethylene terephthalate (PET) layers by RF sputtering using a roll-to-roll process were prepared by Win Optical Technology, Taiwan. To determine a suitable resistance of ITO layers as sensing electrodes of the ITO/PET-EGFET system, the sheet resistances were controlled at 100, 400, and 500 Ω/□. The sheet resistances were examined by four-points probe stage. To define the sensing area of the ITO electrodes, a conventional photolithography technique was used. Due to the low thermal budget tolerance of PET substrates, the temperature of all the baking process in photolithography process was set below 100°C. After the hard baking step, the samples were immersed in aqua regia solution with a volume ratio of 37%-HCl: 67%-$HNO_3$: de-ionized (DI) water (50:3:50) for 10s~~ec~~. Then, the sensing area of the ITO/PET electrode was defined as a circle with a 2-mm radius using the epoxy-resin-type adhesive JU-100 (*KOKI Company Ltd.*) applied by an

automatic x-y stage packager, as shown in Fig.1.

To measure the current-voltage (I-V) characteristics of ITO/PET-EGFET, a Keithley 4200 apparatus was used. A commercial n-MOSFET was chosen as the FET device of the EGFET, and the gate electrode was connected to the ITO/PET electrodes. Then, only the ITO/PET electrodes and a commercial Ag/AgCl reference electrode were immersed in the buffer solutions. All measurement setup were carried out in a Faraday cage at room temperature to keep from light and noise interference. To measure the pH sensitivity, the pH buffer solutions were changed from pH 2 to pH 12. For urea measurement, I-V curve was measured after three minutes as the response time to let the enzyme react with urea. Furthermore, between each measurement, ITO/PET electrodes were kept in 4°C. Figure 2 shows the process flow of enzyme immobilization. After ITO/PET electrode fabrication, 2.5% glutaraldehyde (GA) solution was dripped on ITO membrane. Finally, urease was dripped on the sensing membrane and stored in fridge at 4℃ overnight. The non-immobilized enzyme was rinsed by 5 mM phosphate buffer with 0.1 M NaCl at pH 6. A commercial MOSFET was selected as the transistor of EGFET, and the current-voltage curves were measured using Kethiely 4200.

## Results and Disscusion

To get the pH sensitivity of the ITO/PET-EGFET, the drain to source current –gate to source voltage ($I_{DS}$-$V_{GS}$) curves measured by Keithley 4200 in different pH buffer solutions were shown in Fig. 3. The curves shifted to the positive voltage while the samples were immersed into higher pH value of buffer solutions, which can be explained by the site-binding theory. For each curve, the corresponding voltage at the $I_{DS}$=1 μA is determined as output voltages.

In order to realize the characteristic of different sheet resistances of the ITO/PET sheets, 100, 400, and 500 Ω/□ ITO/PET electrodes were fabricated. The trend of sensitivity and linearity of the ITO/PET-EGFETs with different sheet resistances of 100, 400, and 500 Ω/□ are shown in Fig. 4. For the ITO/PET electrodes with higher sheet resistance, the lower pH sensitivity and lower linearity of calibration curve were observed. The results are similar with other metal oxide, such as anodized AlO and sputtered $SnO_2$ layers, the low resistance or resistivity of the sensing membrane on EGFET were observed the higher and stable pH response. Therefore, the ITO/PET electrode with the sheet resistance of 100 Ω/□ was chosen as the highest potential for applying to the commercial pH sensor, and the further reliability tests and urea detection were evaluated with this condition.

The pH sensitivities of the ITO/PET electrodes treated with and without $NH_3$ plasma were shown in Fig. 5. The pH sensitivities of the samples treated with $NH_3$ plasma were all around 51±4 mV/pH. Only the condition of ITO/PET electrodes treated with $NH_3$ plasma with 100W for 9min shows a lower pH sensitivity. Therefore, to investigate this phenomenon, the surface condition of ITO/PET sheets was analyzed by atomic force microscopy (AFM), as shown in Fig. 6. It indicates that ITO surface were damaged after 9 min plasma treatment. Figure 7 (a) shows the responses of ITO/PET films with $NH_3$ plasma at 100W for 3, 6, and 9 min. The urea sensitivity for ITO/PET sheets with $NH_3$ plasma at 100W for 3, 6, and 9 min were 20.7, 36.3 and 43.3 mV/p$C_{urea}$, respectively. The higher output signal was observed at higher plasma power. It suggests that the larger plasma power is beneficial for nitrogen reaction with ITO film. Figure 7(b) shows the lifetime monitoring based on the samples with $NH_3$ plasma at 100W for 9 min. It shows that the lifetime was larger than 6 days with linearity of over 90%.

## Conclusion

A simple $NH_3$ plasma treatment was successfully developed on ITO membrane to creat the amine bond on the surface for urease immobilization in covalent bonding method. There is no obvious difference in pH sensitivity of all groups expect 9 min plasma treated samples.The results exhibit the urea sensitivity is increased with increasing treatment time.

## Acknowledgements

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## References

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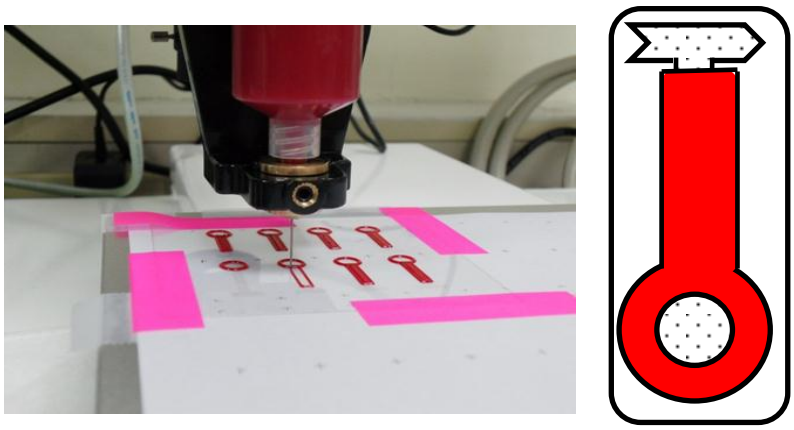


*Fig. 1. ITO/PET electrode sensing area defined by an automatic x-y stage packager.*

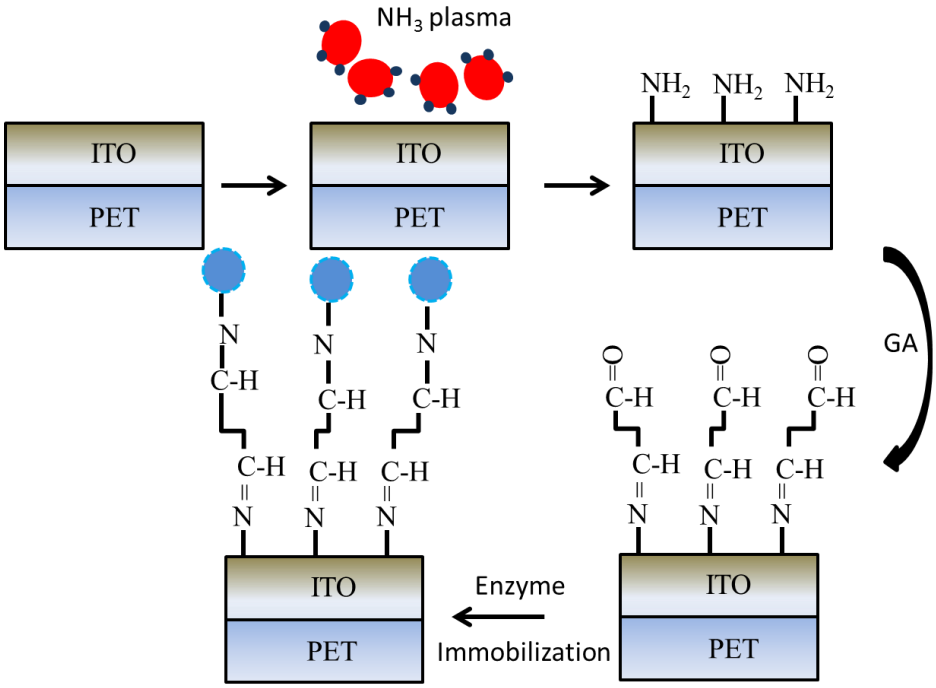



*Fig.2 Schematic of enzyme immobilization process flow.*

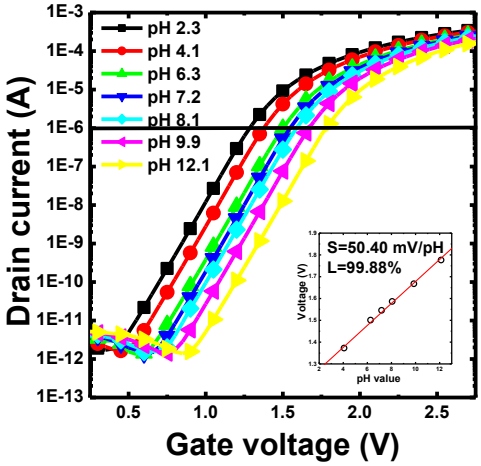



*Fig. 3. $I_{DS}$–$V_{GS}$ curves of 100 Ω/□ITO/PET-EGFET samples measured in buffer solutions ranging from pH 2.3 to pH 12.1.*

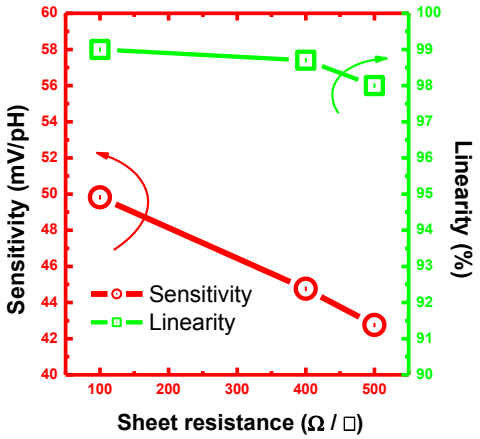



*Fig. 4. Trend of pH sensitivity and linearity of the calibration curve of ITO/PET EGFET samples with sheet resistances ranging from 100 to 500 Ω/□.*

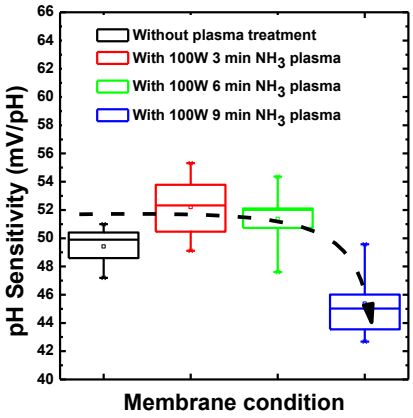



*Fig. 5. Sensitivity of ITO/PET electrode with different $NH_3$ plasma conditions.*

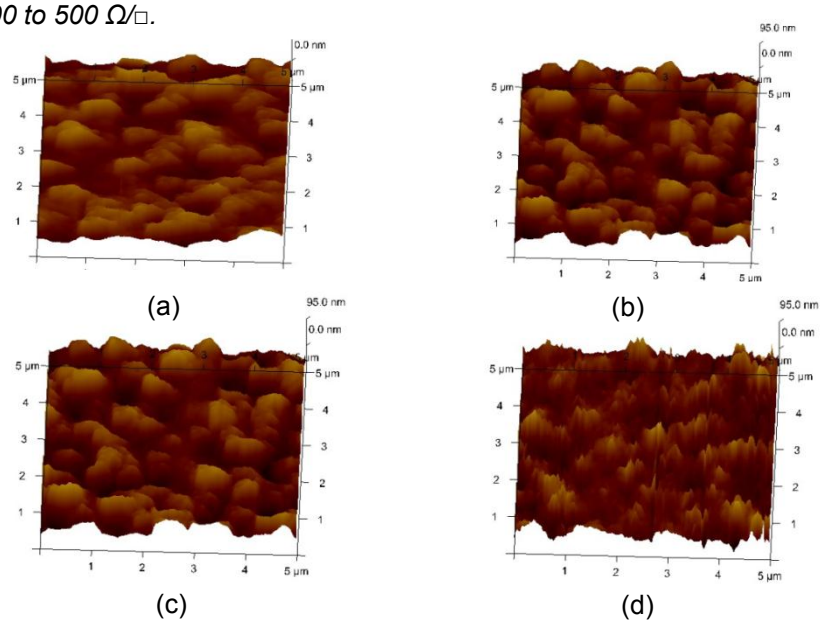
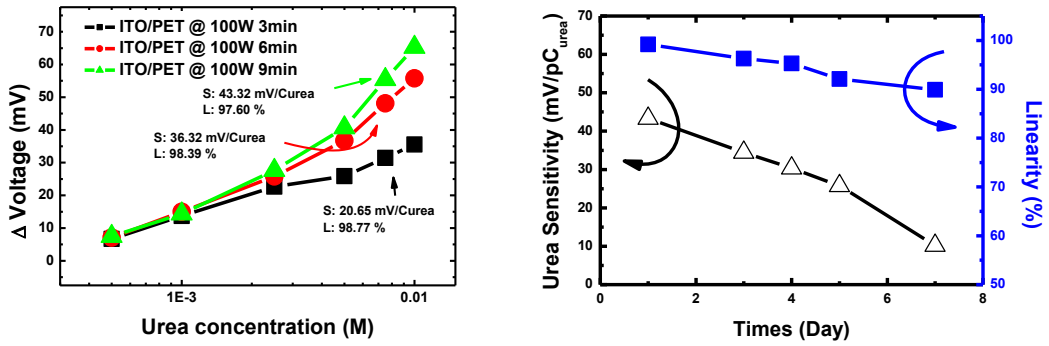



*Fig. 6. AFM image of ITO/PET electrodes (a) without plasma treatment, with $NH_3$ plasma for 100W (b) 3, (c) 6 and (d) 9 min.*




(a) (b)

*Fig.7. (a) Urea-sensing responses of ITO/PET sheets with vairous $NH_3$ plasma condition. (b) Lifetime of ITO/PET samples treated with 100W for 9 min $NH_3$ plasma.*