

Develop urea biosensor based on an ammonium electrode

Po-Yi Chen¹, Ta-Hsiung Cho², Li-Te Yin^{3*}

¹Department of Optometry, Chung Hwa University of Medical Technology, Tainan, Taiwan, ROC

²Department of Optometry, Shu Zen Junior College of Medicine and Management, Kaohsiung, Taiwan, ROC

^{3*}Department of Optometry, Chung Hwa University of Medical Technology, Tainan, Taiwan, ROC

E-mail: leaderyin@gmail.com; Phone: +886958930673

Abstract: Ammonia (NH₃), a kind of compound present in the air or natural water, has the simplest chemical structure among all nitrogen compounds. At a pH below 8, ammonia will interact with water molecule and exist in the form of ammonium ions (NH₄⁺) after dissolved in water. This paper utilizes extended-gate indium tin oxide/polyethylene terephthalate (ITO/PET) ion selective field effect transistors to make durable ammonium ion selective electrode, and investigates influences of each parameter on overall reaction. Results of several experiments show detectable concentration of ammonium ion is 10⁻⁵ ~ 1M, with a linear range of about 0.99 and an average sensitivity of 55.09 mV / pNH₄⁺. Buffer solution is measured by virtue of PB and Tris. Use of Tris buffer can make measurement results tend to be stable. Experimental range of urea concentration spread is 1mg/dl~8mg/dl. Such spread result indicates electrode reaction under low urea concentration is more sensitive than common sensor and limit of detection is lower. In terms of human urea nitrogen detection, this method can be used for diluting normal human urea or blood to an optimal detectable concentration and thus obtaining the most sensitive and reliable reaction result.

[Po-Yi Chen, Ta-Hsiung Cho, Li-Te Yin. **Develop urea biosensor based on an ammonium electrode.** *Life Sci J* 2014;11(8):707-710] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 104

Keywords: ITO/PET, EGFET, urea biosensor

1. Introduction :

In the nature, excessive ammonia content will lead to eutrophication of water and further cause imbalance of aquatic ecosystem. In human body, normal urea level in plasma is 18-36 mg/dl. Clinically, urea measurement is usually represented by its nitrogen content and called urea nitrogen. 1 mg/dl urea nitrogen is equivalent to 2.14 mg/dl urea concentration. Urea nitrogen can present difference in urea concentration as a result of different human status. Therefore, measuring urea content in blood is clinically significant.

Enzyme biosensor is a kind of biosensor not only substantially and deeply investigated by research field but also actively discussed in electrochemical analysis domain. This biosensor can utilize conductor or semiconductor as base electrode, fix bioactive substance onto electrode surface by chemical modification and enzyme immobilization, and cooperate with transducers to convert variable quantity of output voltage into meaningful electronic signal through various physical and chemical conversion modes.

Along with advancement in technology of metal-oxide-semiconductor field effect transistor (MOSFET), sensors derived from MOSFET are gradually applied into diverse researches. For instance, Ion-Sensitive Field Effect Transistor (ISFET), firstly published by P. Bergveld in 1970, is a product generated through integrating electrochemistry with

microelectronics and possesses both functions of ion selective electrode and characteristics of field effect transistor [1]. Due to appearance of field effect transistor sensor and constant improvement in technology and elements, ISFET is applicable to diversified fields including human, agricultural and chemical applications. Subsequently, some people propose new ISFET such as hydrogen-ion-sensitive SnO₂ [2] and ion-sensitive extended gate field effect transistor (EGFET), and the latter is advantageous in low cost, easy packaging and high stability and thus suitable for point-of-care testing (POCT) [3-5]. As a result of above-mentioned merits, there exist extensive domestic and foreign researches on ISFET, and sensor develops towards being miniaturized, volumetric, multi-functional, intelligent and systematic.

Ammonium-ion-selective electrode can be used to make biosensors including five substrate enzymes: Urease, Creatinine Deiminase, Amino Acid Oxidase, Asparinase and Uric Acid, which can generate ammonium ions in reaction. Therefore, it's apparently that there're many enzyme sensors based on ammonium ion, among which biosensors based on ammonium-ion-selective electrode have optimal sensing properties.

Presently, two kinds of electric-potential ammonium-ion-selective electrodes are commercially available. The first is gaseous ammonium ion sensor composed of glass film and selective breathable film.

For use of such sensor, alkaline solution must be added to sample solution so as to ensure release of ammonium gas, which will permeate selective breathable film and then utilize glass film sensor to conduct detection.

The second electrode is a sensor constituted by PVC film, with Nonactin as the carrier. Such sensor can detect ammonium ions without any in-advance treatment. It is broadly used for monitoring ammonium ions in waste water because of its advantages more than gaseous ammonium ion sensor such as cost effectiveness, easy operation and usage, and no need for pH value adjustment of sample solution. However, voltage response of such electrode is influenced by anion surfactant and hydrophobic substance interference [6, 7].

Actually, ammonium-ion-selective electrode composed of PVC film has a short life span in waste water probably because the sensor is degraded due to a contact with sewage containing organic compounds. Organic compounds like ionic surfactant and alcohol may eliminate sensing function of the film. In fact, such phenomenon can be observed in all sensors based on PVC film.

Therefore, reason for degradation of sensing film based on PVC is likely that the sample solution contains some organic compounds such as organic anion, ionic surfactant and alcohols [8]. According to previous researches, ammonium-ion-selective electrode based on urea biosensor features shorter response time as well as wider limit of detection and linear range than pH urea electrode [9].

This paper utilizes extended-gate ITO/PET ion selective field effect transistors to make durable ammonium ion selective electrode, and investigates effects of each parameter on overall reaction.

2. Experimental:

2.1 Preparation of windable sensor

Lay ITO/PET on the ovenware, cut the copper wire, on one end of the copper wire with elargol, place it at the upper third of the ITO/PET, and then put the ovenware into the oven. After baking under 110°C for about 20 minutes, take the ovenware out, and wrap the external of ITO/PET with Epoxy to leave a 3 X 3 mm window. A windable sensor is made. Then, use measurement infrastructure to measure the packaged windable sense element of ITO/PET for the purpose of determining its linearity and sensitivity. The best sensitivity of element is 54~59 mV/pH. Optimal linearity of windable sensor is greater than 0.999.

2.2 Preparation of ammonium responsive electrode

Ammonium responsive PVC-COOH device were obtained by casting doped films from tetrahydrofuran (THF). The following chemicals were used for preparing the PVC selective solutions: PVC(33%),

Bis(2-ethylhexyl) sebacate(66%), Tris(hydroxymethyl)-aminomethane (20 mmol/l), Nonactin (1%). Ammonium responsive PVC-COOH device were obtained by casting doped films from tetrahydrofuran (THF). The following chemicals were used for preparing the PVC selective solutions: PVC (33%), Bis(2-ethylhexyl) sebacate (66%), Tris(hydroxymethyl)-aminomethane (20mmol/l), Nonactin (1%). Blend and dissolve above materials 100ml THF according to above proportion, drip 4uL on ITO/PET sensing gate, keep a 12-hour standing under room temperature, and ammonium responsive electrode is made.

2.3 Preparation of ammonium-ion urea sensor

After NH₄Cl concentration experiment is completed and sensitivity and linearity of ammonium electrode are confirmed to meet requirements, fix a layer of urease on ammonium responsive film, wait for an hour at 4°C, and ammonium-ion urea sensor needed by the experiment is made.

3. Results and discussions:

3.1. Characteristics of ammonium-ion sensor

First of all, concentration range that can be detected by ammonium carrier must be determined in this paper. The present study uses ammonium ion sensor to measure NH₄Cl with a concentration of 10⁰M~10⁻⁵M. Data acquired through measuring each kind of NH₄Cl solution of different concentration for minute are mainly used. Lab View software is used to capture responsive voltage measured under each concentration. Next, Origin50 is utilized to generate calibration curve of responsive voltage. As illustrated in figure 4-1, the sensor has a sensitivity of 55.0 mV/decade and a linearity (R²) of 0.998 when measuring ammonium solution.

3.2 Influence of buffer solution on urea detection

Experimental result may present difference in data due to use of different buffer solutions. Therefore, it's necessary to find out buffer most suitable for the present experiment so as to avoid responsive voltage error obtained during experiment. For urea with concentration of 8, 12, 16, 24, 32mg/dL, phosphate buffer (PB, 20mM, pH7.4) and Tris(hydroxymethyl)-aminomethane (Tris, 20mM, pH7.4) buffer are used to conduct experiment. As shown in Fig. 2, in use of phosphate buffer, it's impossible to observe the reaction, during which urea elevates sensor voltage after hydrolysis by urease. When Tris buffer is adopted, we can obviously observe increase in sensor voltage resulting from change of urea concentration. Furthermore, reaction trend for concentration above 8mg/dl presents saturated condition. Therefore, this study will use Tris to perform following experiments and choose 8mg/dl as the highest limit of detection for

urea concentration.

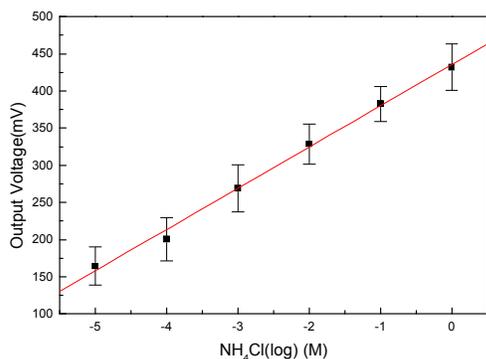


Fig.1 Calibration curve of ammonium-ion sensing electrode

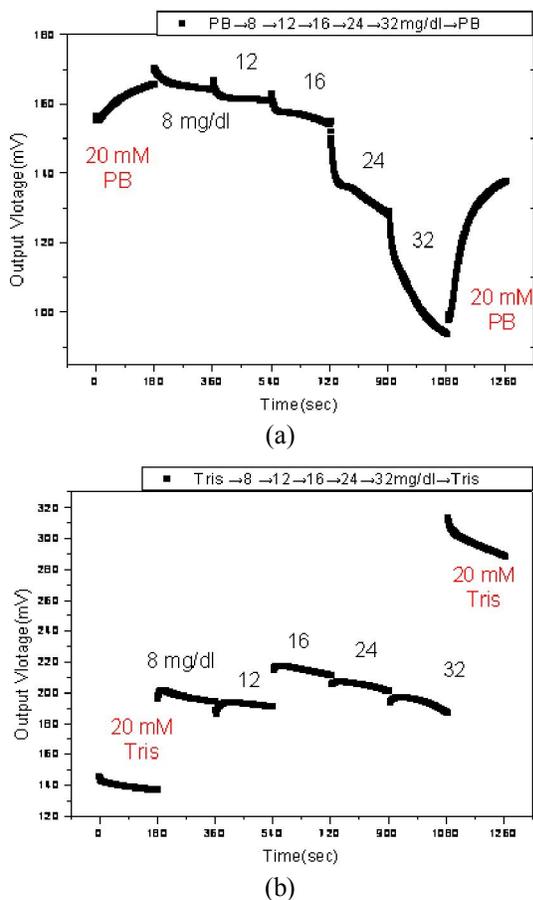


Fig.2 Changes in responses to urea concentration for use of different buffer solutions (a) Tris buffer. (b) phosphate buffer

3.3 Influence on urea response after addition of NH_4Cl into buffer solution

To make the sensor have stable reaction value, in consideration of steady reaction of the device in NH_4Cl solution, this experiments uses Tris solutions with

$\text{NH}_4\text{Cl}(10^{-5}\text{M})$ or without NH_4Cl to conduct urea detection, and observes reactions. The reaction trend is shown in figures 4-5(A)(B). Compared with buffer without NH_4Cl , buffer containing NH_4Cl has greater and obvious response to urea concentration (B). Figure (C) is the calibration curve of figure (B). Therefore, subsequent experiments will add NH_4Cl to the buffer solution. However, these experiments indicate no output voltage is observed when urea concentration is less than 0.1mg/dl. Consequently, this paper will use 0.1mg/dl as the lowest limit of detection.

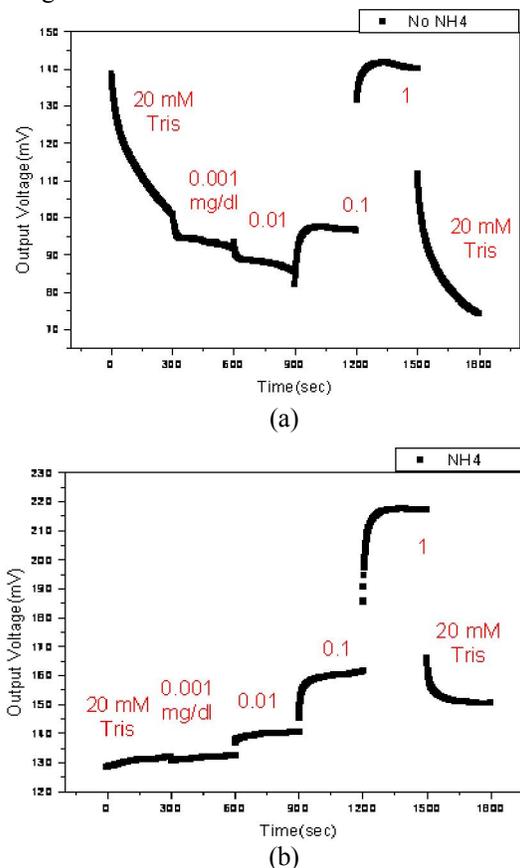


Fig.3 A comparison of urea concentration reactions for buffer solution (a) with NH_4Cl and (b) without NH_4Cl

3.4 Selectivity of urea concentration range response

To know limit of detection (LOD) and dynamic range of ammonium-ion urea sensor proposed in this paper, we carry out spread experiments with urea detection concentration of 0.01, 0.1, 1, 1.33, 1.66, 2, 3, 4, 5, 6, 7, 8mg/dl. As illustrated in figure 4, the instrument LOD is calculated from the mean and standard deviation of the replicate blank readings. LOD of this sensor is 0.1mg/dl. In case of urea concentration greater than 5mg/dl, there is no obvious increase in signals of the sensor. Therefore, dynamic range of the sensor is 0.1mg/dl~5mg/dl.

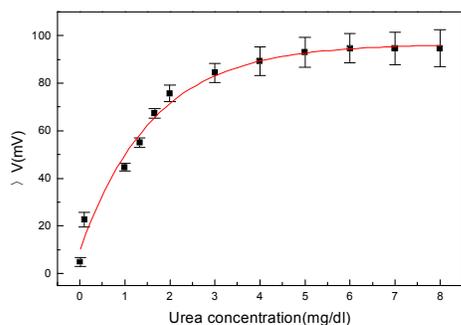


Fig.4 Calibration curve of urea sensing electrode

4. Conclusions:

A series of experimental design and plans aim to develop a kind of highly-sensitive ammonium-ion-selective electrode, which is found by the experiment to have higher sensitivity and lower LOD than common urea sensor. Optimal concentration range of ammonium-ion sensing electrode is $10^0\text{M}\sim 10^{-5}\text{M}$ (5,349mg/dl \sim 0.05349mg/dl). After calibration, it has a sensitivity of 55.0 mV/decade and a linearity of 0.99. Experiment of buffer selection shows electric voltage obtained in Tris buffer is steadier than PB buffer, and that addition of NH_4Cl can stabilize urea detection. Moreover, compared with ITO/PET sensors presented in other literatures, LOD of common urea biosensors (Urease/ITO/PET) is 10mg/dl \sim 80mg/dl but LOD of ammonium-ion sensing electrode (Urease/Nonactin-PVC/ITO/PET) reaches 0.1mg/dl \sim 5mg/dl, indicating a significant enhancement.

This study investigates development of ammonium-ion-selective electrode, with an ultimate purpose of development urea biosensors and applying it in inspection of natural water source. Main origin of ammonium ions in natural water is the rainfall, with a concentration of 20mg/dL approximately. Over-high concentration of ammonium ions may cause eutrophication of water and imbalance of aquatic ecosystem. For example, for protecting fish, maximum allowable concentration of ammonium ions in the water is 4 \sim 20mg/dL in Europe. Therefore, knowing level of ammonium ions in water is very important and highlights importance of the sensor. In terms of human body, normal urea concentration in plasma is about 18-36 mg/dL. In the present experiment, the best detectable concentration of the sensor is 1mg/dL \sim 2mg/dL. Therefore, blood should be diluted to

within such sensitivity range in order to get the best response and reliable result. Actually, value of the ammonium-ion urea sensor is more obvious in natural water because it has lower LOD. Quantity of urea residual in natural water is very small, so sensor with lower LOC will have a greater value in use.

Corresponding Author:

Li-Te Yin M.D.

Department of Optometry, Chung Hwa University of Medical Technology, Tainan, Taiwan, ROC

E-mail: leaderyin@gmail.com

Phone: +886958930673

References

1. P.Bergveld, Development of an ion sensitive solid-state device for neurophysiological measurements, *IEEE Trans. Biomed. Eng.*, 17 (1970) 70-71.
2. Li-Te Yin, Jung-Chuan Chou, Wen-Yaw Chung, Tai-Ping Sun and Shen-Kan Hsiung, Separate structure extended gate H^+ -ion sensitive field effect transistor on a glass substrate, *Sensors and Actuators B* 71(2000)106-111
3. Li-Te Yin, Ya-Ting Lin, Ying-Chen Leu, Chih-Yu Hu, Enzyme immobilization on nitrocellulose film for pH-EGFET type biosensors, *Sensors and Actuators B*, 148(2010)207-213
4. Yin LT, Wang HY, Lin Y, Huang WC. "A Novel Instrumentation Circuit for Electrochemical Measurements". *Sensors* 2012 ; 12 : 9687-9696.
5. Po-Yi Chen, Li-Te Yin, Ming-Der Shi, Yi-Chieh Lee, Drift and light characteristics of EGFET based on SnO_2/ITO sensing gate, *Life Science Journal* 10(2013)3132-3136.
6. Anthony J Frennd, Gwilym J. Moody, J. D. R. Thomas, B. J. Birch, Study of calcium ion-selective electrode in the presence of anionic surfactants. *Analyst* 108(1983)1072-1078.
7. Yasuhisa Shibata, Kunio Hirota; Hiroyuki Miyagi; Yoshinori Takata, 1993. Interfering substances in the analysis of patient serum samples with dialysis by chloride ion-selective polymeric membrane electrodes based on methyltridodecylammonium salt. *Bunseki Kagaku* 42(1993)265-271.
8. A. Hulanicki, M. Trojanowicz and E. Pobozy, Effect of surfactants on the response of ion-selective electrodes with poly(vinyl chloride) membranes. *Analyst* 107(1982) 1356-1362.
9. N.H. Chou, J.C. Chou, T.P. Sun, S.K. Hsiung, Study on the disposable urea biosensors based on PVC-COOH membrane ammonium ion-selectiveelectrodes, *IEEE Sens. J.* 6 (2006) 262-268.