Urea Amperometric biosensor based on entrapment immobilization of urease onto a nanostructured polypyrrol and multi-walled carbon nanotube

Hamide Amani¹, Afshin FarahBakhsh², Sina Aghili³

¹Department of Chemical Engineering, Quchan Branch, Islamic Azad University, Quchan, Iran.
²University of Applied Science & Technology, Shirvan Center, Shirvan, North Khorasan, Iran
³Department of Chemical Engineering, Quchan Branch, Islamic Azad University, Quchan, Iran.

*Email: sinaaghili@hotmail.com,

ABSTRACT

A biosensor is an analytical tool that comprises two essential components—an immobilized biocomponent, in intimate contact with a transducer that converts a biological signal into a measurable electrical signal. An amperometric biosensor based on surface modified polypyrrole (PPy) has been developed for the quantitative estimation of urea in aqueous solutions. The incorporation of urease (Urs) into a bipolymeric substrate consisting of PPy was performed by entrapment to the polymeric matrix, PPy acts as amperometric transducer in these biosensors. To increase the membrane conductivity, multi-walled carbon nanotubes (MWCNT) were added to the PPy solution. The entrapped MWCNT in PPy film and the bipolymer layers were prepared for construction of Pt/PPy/MWCNT/Urs. Two different configurations of working electrodes were evaluated to investigate the potential use of the modified membranes in biosensors. The evaluation of two different configurations of working electrodes suggested that the second configuration, which was composed of an electrode-mediator-(pyrrole and multi-walled carbon nanotube) structure and enzyme, is the best candidate for biosensor applications.

Keywords: urea biosensor, urease, polypyrrole, multi-walled carbon nanotube.

1. INTRODUCTION

A biosensor is an analytical device based on the direct spatial coupling of an immobilized biologically active compound with a compatible transducer, which converts the biochemical signal into a quantifiable electrical signal. This synergistic combination of biotechnology and electronics has given rise to a generation of technology called biosensor technology. Enzymes, whole cells, tissues, receptors, antibodies constitute the biological component of the biosensor. The enzymes are highly specific, so find extensive applications in biosensors [1].

Several methods for enzyme immobilization have been proposed, and these methods can be classified into physical and chemical methods. Physical methods, which include adsorption, encapsulation and entrapment, are simple, but the interactions between the support and the enzymes are relatively weak, leading to enzyme leakage. Entrapment in a conductive matrix has also been used. Other techniques, including entrapment in an organic polymer, entrapment on carbon-polymer electrodes and the solgel method, have also been developed. Chemical methods are relatively complex, but due to the formation of covalent bonds between the enzyme and the substrate, the stability of enzyme immobilization should be higher than that obtained when using physical methods [2]. The immobilization of enzymes in polymeric matrices is an alternative approach for preparing biosensors, which have been used to detect different types of analytes in small-volume samples [8].

A suitable choice of a polymer matrix for the immobilization of urease is polypyrrole (PPy) a highly stable, biocompatible polymer that can be prepared electrochemically at relatively low potentials in non-aqueous and aqueous media in a wide range of pH [8]. Recently nanoparticles enhancing enzyme immobilization technique have become widespread. The using of carbon nanotubes (MWCNT) as mediators of the electron transfer from the enzyme molecules to the electrode surface is often applied. Their unique electronic properties suggest that
MWCNT have the ability to promote the electron transfer reactions of biomolecules in electrochemistry. Their mechanical properties, high aspect ratio, electrical conductivity and chemical stability make MWCNT perfect for a wide range of applications that include fabrication of urease biosensors.[9]

Urea is a biomolecule which is excreted by kidney as an end product of protein metabolism [3]. The level of urea in blood serum is the best measurement of kidney function and staging of kidney diseases. The normal urea level in serum ranges from 15 to 40 mg/dL (i.e., 2.5–7.5 mM) [4]. An increase in urea concentration causes renal failure (acute or chronic), urinary tract obstruction, dehydration, shock, burns and gastrointestinal bleeding, whereas a decrease in urea concentration causes hepatic failure, nephrotic syndrome, cachexia (low-protein and high carbohydrate diets) [5]. Hemodialysis is an important clinical procedure for the removal of toxic biological metabolites in patients with end-stage renal disease. A hemodialyzer is the semipermeable membrane, which allows for selective transport of low molecular weight biological metabolites from blood [3]. The hydrolysis of urea, which can be catalyzed by Urease (Urs), yields a typical increase in pH of the medium according to the following reaction (reaction (1)) [8]:

\[
\text{NH}_2\text{CONH}_2 + 3\text{H}_2\text{O} \rightarrow \text{HCO}^- + 3\text{OH}^- + 2\text{NH}_4^+ \quad (1)
\]

The aim of this study is to investigate the use of all solid-state contact type PPy and PPy/MWCNT membrane electrodes for the construction of enzymatic sensors based on entrapment immobilization of urease to nanostructured electrodes proposed for urea. Enzyme (urease) was deposited onto all solid-state contact PPy membrane via cyclic voltammetric method. Both types of electrodes were used for the assay of blood urea in clinical samples. Furthermore, the immobilization on PPy/MWCNT was used to design a new urea biosensor for estimation of urea in biological samples.

2. Experimental

2.1. Reagents and Chemicals

Pyrrole (Py), 98% from Sigma-Aldrich; Urease (Urs), EC 3.5.1.5, from jack beans, (Type III, 20 KU·g⁻¹ solid) from Sigma-Aldrich; Multi-walled carbon nanotubes (MWCNT, with 90% purity); KH₂PO₄ and K₂HPO₄ (chemicals for phosphate buffer); KCl, sulfuric acid and Aceton were purchased from Merck. All reagents were of analytical grade. All solutions were prepared using deionized water.

2.2. Instrumentation

Cyclic voltammetric, amperometric measurements and electropolymerization of Py monomers on working electrode surface were carried out with the AMEL Instruments (Model 7050 POTENIOSTAT, Italy) and three electrode electrochemical cell: a platinum disk electrode (0.5 cm² area) as a working electrode, platinum wire as a counter electrode and a saturated calomel (SCE) or Ag/AgCl electrodes as reference electrodes were used both in the cyclic voltammetric and amperometric measurements.

2.3. Cleaning of the Working Electrode Surface

The working electrode was mechanically polished with 0.05 and 0.3 μm alumina, rinsed with distilled water, acetone and once again with water and electrochemically pre-treated by potential cycling (between -0.21 and +1.19 V versus Ag/AgCl) in 1 M H₂SO₄ at a scan rate of 0.05 V/s until a steady state voltammogram was obtained.

2.4. Preparation of Pt/PPy/Urease Biosensor

The electropolymerization of Py was carried out in 0.01 M KCl as supporting electrolyte, containing 0.1 M NaCl and 0.4 M Py monomer solution. The final concentration of urease in this solution was 0.1%. The working electrode potential was cycled in the potential range from -0.7 to +1.2 V at a scan rate of 0.05 V/s for 30 cycles.

2.5. Preparation of Pt/PPy/MWCNT/urease biosensor

The electropolymerization of Py was carried out in 0.01 M KCl as supporting electrolyte, containing 0.1 M NaCl and 0.4 M Py monomer solution. 0.002 g MWCNT were added and the mixture was homogenized by sonication for 1.5 h. Then urease was added to this solution to a final concentration of 0.1%. The working electrode potential was cycled in the potential range of -0.7 to +1.2 V at a scan rate of 0.05 V/s for 30 cycles.

3. Results and Discussion

3.1. Performances of the Pt/PPy/Urease electrode to urea detection
Fig. 1 shows the CV of the electrochemical cell using Pt/PPy/Urease electrode at a constant 0.05 V/s scan rate in 0.1 M phosphate buffer solution at pH= 7.0. The current of the electrochemical was \(2.4 \times 10^{-3}\) A.

The electrochemical response of the assembled multilayers system was studied by capacitance measurements. Fig.2 shows the amperometric response of Pt/PPy /urease biosensor for different urea concentration and the steady-state current dependence calibration curve for the urea concentration (C) ranging from 0.025 to 0.1mM. An amperometric linear response \((r^2 =0.987)\) was observed with the successive addition of urea to the phosphate buffer solution containing 0.1M KCl as the electrolyte.

### 3.2. Performances of the Pt /PPy/MWCNT/urease electrode to urea detection

The multi-layered urease biosensors were prepared by methodic described above. MWCNT were incorporated within the growing PPy film for maintaining its electrical neutrality. This is completely understandable since added MWCNT improve electrical conductivity of the polymer film, the film was more porous and the diffusion of the substrate was more intensive. The current of the electrochemical was \((5.02 \times 10^{-3}\) A).

Fig.4 shows the amperometric response of Pt/PPy/MWCNT /urease biosensor for different urea concentration and the steady-state current dependence calibration curve for the urea concentration ranging from 0.025 to 0.1 mM. An amperometric linear response \((r^2 =0.997)\) was observed with the successive addition of urea to the phosphate buffer solution containing 0.1M KCl as the electrolyte.

### 3.3. The Comparison of the Pt /PPy/urease and Pt /PPy/MWCNT/urease electrodes to urea detection

The comparison of the two urea calibration curves showed that the curve slope of the Pt/PPy/MWCNT/urease electrode was larger and this electrode had the greatest sensitivity. This was due to the incorporation of MWCNT in deposited PPy film of the electrode. This is completely understandable since added MWCNT improve electrical conductivity of the polymer film, the film was more porous and the diffusion of the substrate was more intensive. The sensitivity of Pt/PPy/ urease electrode was on the second place. Fig.3.

### 4. Conclusion

A new type of urea biosensor based on a Pt/PPy/MWCNT/Urs bioelectrode was successfully fabricated and evaluated. Urs was entrapment onto the nanoporous film modified by multi-walled carbon nanotubes. The Pt/PPy/MWCNT electrode surface offered a high level of enzyme immobilization leading to a highly stable Pt/PPy/MWCNT/Urs bioelectrode. The urea biosensor showed a linear current response to the urea concentration ranging from 0.025 to 0.1mM. This new type of urea biosensor has demonstrated superior performance compared with those reported previously, including a longer shelf life, higher sensitivity, wider range of detection limit, and shorter response time.

### Acknowledgement

The authors are grateful to the Research Council of the Quchan Islamic Azad University for the financial support of this research.

### References


**CITATION**

**Fig. 1** Cyclic voltammogram of the Pt/PPy/urease electrode in 0.1 M phosphate buffer solution at pH = 7.0

**Fig. 2** Amperometric response of Pt/PPy/Urs bioelectrode with the urea concentration ranging from 0.025 to 0.1 mM at working potential of -0.7 to 1.2 V vs. Ag/AgCl and scan rate of 0.05 V/s

**Fig. 3** Cyclic voltammogram of the Pt/PPy/MWCNT/urease electrode in 0.1 M phosphate buffer solution at pH = 7.0

**Fig. 4** Amperometric response of Pt/PPy/MWCNT/Urs bioelectrode with the urea concentration ranging from 0.025 to 0.1mM at working potential of -0.7 to 1.2V vs. Ag/AgCl and scan rate of 0.05 V/s

**Fig. 5** Comparison of Amperometric response of Pt/PPy/MWCNT/urease (◊) and Pt/PPy/urease (□) bioelectrod with the urea concentration ranging from 0.025 to 0.1mM vs. Ag/AgCl and scan rate of 0.05 V/s
Fig. 1
Fig. 2

\[ R^2 = 0.9872 \]
Fig. 3
Fig. 4

$R^2 = 0.9977$

I/A vs. C/mM graph.
Fig. 5

$R^2 = 0.9977$

$R^2 = 0.9872$