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POLYMER MEMBRANE BIOSENSOR BASED ON POTENTIOMETRIC SENSING OF AMMONIUM AND UREA

AMONYUM VE ÜRENİN POTANSİYOMETRİK ALGILANMASINA DAYALI POLİMER MEMBRAN BİYOSENSÖRÜ

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A poly(vinyl chloride) (PVC) matrix nonactin based ammonium selective membrane electrode was developed as sensors for ammonium (NH_4^+) as well as for urea determination after jack bean meal was immobilized.

This NH_4^+ selective electrode exhibited Nernstian response over the linearity range $9.5x10^{-5}$ – $1.0x10^{-1}$ M with slope of 59 mV/decade in the standard NH_4^+ solutions. The linearity range was found $9.0x10^{-5}$ –$1.0x10^{-1}$ M with slope of 40 mV/decade with enzyme electrode in the standard urea solutions.

As the nonactin based ammonium selective electrode suffers from interference of potassium and sodium ions in the urea solution, the interference effect of sodium and potassium ions has been investigated.

Polivinil klorür (PVC)-nonaktin matriksine dayalı amonyum seçimli membran elektrot amonyum için aynı zamanda jack bean meal immobilize edildikten sonra da üre tayini için sensör olarak geliştirilmiştir.

Bu amonyum seçimli elektrot, amonyum standart çözeltilerinde, $9.5x10^{-5}$ –$1.0x10^{-1}$ M doğrusal aralığında derişimdeki on misli değişime karşılık 59 mV eğim değeriyle Nernst yanıtına yakın değer göstermektedir. Enzim elektroduyla standart üre çözeltilerinde doğrusal aralık, derişimdeki on misli değişime karşılık 40 mV'luk eğim değeriyle $9.0x10^{-5}$ –$1.0x10^{-1}$ M olarak bulunmuştur.

Nonaktine dayalı amonyum seçimli elektrotla potasyum ve sodyum iyonlarının üre çözeltisindeki girişimlerinden dolayı, sodyum ve potasyum iyonlarının girişim etkisi incelenmiştir.

Keywords : *Ammonium; Urea; Jack Bean Meal; Ion-selective Electrode; Biosensor*

Anahtar kelimeler: *Amonyum; Üre; Jack Bean Meal (Fasulye Tozu); İyon Seçimli Elektrot; Biyosensör*

Introduction

Ion-selective and othed potentiometric membrane electrodes are in view of the great importance to chemistry, biology and medicine. Polymer membrane type ion-selective electrodes (ISEs) are now used routinely within biomedical instruments to measure clinically important ions (e.g. Na^+, Li^+, K^+, etc.) in diluted whole blood (1,2).

A new trend in the development of biosensors is the utilization of novel biological materials as biocatalytic layers (3). Enzyme electrodes are important type of electrochemical biosensor that have application in clinical diagnostics, biomedical research, process monitoring and artificial organs. Rapid growth in

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enzyme immobilization techniques and the need of faster and accurate determination of metabolites in blood has stimulated interest in finding adequate materials and techniques for improving the features of enzyme electrode.

The determination of urea in clinical and other areas is of great importance. Conventional methods for urea determination are mostly based either on direct colour reaction or on the spectrophotometric measurement of ammonia produced by the delicate procedures and assay times are rather long because of the several reactions involved(4). The spectrophotometric methods are difficult with coloured samples and for biological fluids. Alternative approaches based on electrochemical sensors have been developed for urea. These usually involve the electrochemical detection of ammonia produced in the presence of urease(5).

Several enzymatic methods have been reported for urea determination (3,6). The use of less expensive whole tissue sections of plant or mammalian is a possible alternative to high-cost isolated enzymes. Deng et al. (3), have reported old soybean meal electrode sensitive to urea.

Several authors have used ammonia gas electrodes for urea determination and although these probes are still widely used they suffer several limitations, including slow response time especially at low gas concentration, pH adjustment problems and volatile bases that may be present in a sample (7).

PVC matrix nonactin based ammonium selective membrane electrodes have been widely described by many authors and used as sensors for ammonium(8), as well as for urea analysis (7,9).

In this study, the conventional type of PVC matrix nonactin based ammonium ion selective membrane electrodes have been prepared and used as sensors for ammonium as well as urea analysis. For NH_4^+ determination, Lui et al. (10) studied solid-state ion-selective electrodes which retained a lipophilic silver-ligand complex together with free ligand and nonactin. Palleschi et al.(7) and Alegret et al. (11) also prepared solid-state PVC matrix membrane electrode which had the advantage of eliminating the internal filling solution. In our study, precision of the slope has been evaluated with the same electrode before and after immobilization of urease which has not been studied throughly. We evaluated an ammonium ion selective electrode as sensor for the ammonium ions liberated in the enzyme urease reaction. The characteristic of this electrode was that the ammonium ion selective membrane is prepared directly on PVC tube. Moreover, when such electrode is assembled as an urea sensor, the enzyme can be immobilized onto the PVC membrane. This allows a more regular diffusion of ammonium ions produced by the urease reaction, that occurs only on the electrode surface, and improves the response time and the reproducibility of the electrode.

Materials and Methods

Apparatus and Reagents

The e.m.f measurements were done at room temperature with an Orion 601 A digital pH meter. The reference electrode was an Orion Ag/AgCl electrode. Jack bean meal which contains urease (E.C. 3.5.1.5. from Jack beans as a crude urease) was from Sigma. All other reagents were of analytical purity. Ammonium chloric, urea, trizma base (Tris), hydrochloric acid, glutaraldehyde, nonactin, PVC, tetrahydrofurane (THF) were obtained from Sigma; Dioctlysebacate (DOS) from Fluca. The dialysis membrane (Dialysis tubing-visking size 10-1 ¼") was from Medicell International LTD.

Construction of the Ammonium Electrode

PVC tube was used for construction of electrode body. The dimension of the electrode body was as follows; internal diameter 8 mm, external diameter 12 mm. DOS was used for binding the ionophore to the PVC matrix. The composition of the membrane was as : 1.4 mg nonactin, 30 mg PVC, 60 mg DOS, dissolved in 2 mL THF. The membrane was ready for use after evaporating THF.. This membrane was replaced on PVC tube by a mixture of PVC, DOS, THF (10 mg:26 mg:1 mL). Internal filling solution of 0.01 M NH_4Cl to ammonium selective membrane electrode was used. Ag/AgCl was used as the inner reference electrode.

Construction of the Enzyme Electrode

10 mg of the Jack bean meal was spread evenly over the entire exposed portion of the ammonium selective membrane electrode; 25 µL of Tris-HCl buffer (0.05 M, pH 7.4) was added to membrane, and the Jack bean meal-buffer mixture was stirred with a small rod. After the meal slurry had the consistency of a uniform paste, 15 µL of 2.5% glutaraldehyde was added, and the suspension was quickly stirred and left to react at room temperature for 15 min. The modified membrane then was immersed in distilled water for 15 min, followed by immersion in 0,1 M glycine solution for 15 min to eliminate excessive glutaraldehyde excess from the membrane. After immersing in a buffer, the membrane electrode was ready for use.

When used as an urea electrode the sensor was assembled with the enzymatic membrane directly in contant with the PVC membrane, with a dialysis membrane fbr external protection (7). Schematic diagram of the enzyme electrode is shown in Fig. 1.

Measurement Procedures

Standard NH_4Cl solutions in the range of $1.0x10^{-6}$-$1.0x10^{-1}$ M were prepared in Tris-HCl buffer (0.05 M, pH7.4). Before the urease was immobilized by glutaraldehyde, ammonium measurements were run by immersing the electrode in different concentrations of the standard NH_4Cl solutions. When the electrode potentials attained the stable value, it was recorded. Calibration plots were drawn.

Standard urea solutions in the range of $1.0x10^{-6}$-$1.0x10^{-1}$ M were prepared in distilled water. After the enzyme was immobilized on the electrode for urea determination, the same procedure described above was applied.

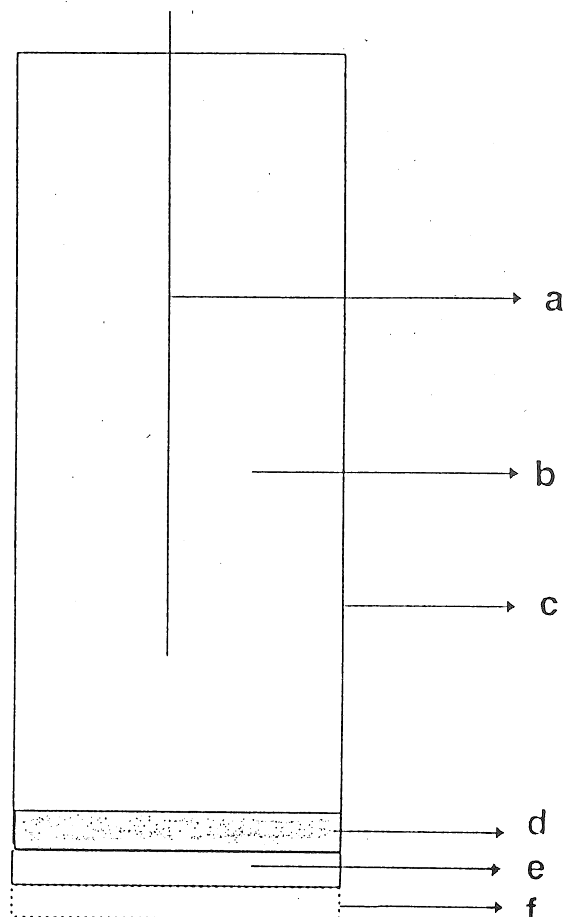
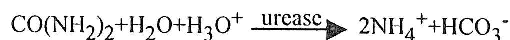


Fig.1. Schematic diagram of the nonactin based electrode:(a) Ag/AgCl; (b) 0.01 M NH_4Cl solution; (c) PVC tube; (d) Nonactin matrix; (e) Jack bean meal enzyme membrane; (f) Dialysis membrane

Results and Discussion

The principal reaction of jack bean meal to urea is:



$$CO(NH_2)_2+H_2O+H_3O^+ \xrightarrow{urease} 2NH_4^+ + HCO_3^-$$

Typical calibration plots for the ammonium selective membrane electrode and the urease enzyme electrode were shown in Fig. 2. As seen in Fig. 2a and 2b, the standard deviation of NH_4^+ electrode is smaller than that of urea electrode. This may be due to enzyme immobilization.

The main electroanalytical data for characterisation of the ammonium membrane electrode and the urea electrode (slope, response time, linearity range and detection limit) were summarized in the Table.

Table. Characterisation of ammonium and urea electrodes

	Ammonium sensor	Urea sensor
Slope (mV/decade)	59±3*	40±1*
Response time (min)	2-5	15
Detection limit (M)	$4x10^{-5}$	$6x10^{-5}$
Linearity range (M)	$9.5x10^{-5}$-$1x10^{-1}$	$9.0x10^{-5}$-$1x10^{-1}$

*n= 3 determinations were taken with the same electrode

The slopes were found to be 59, 40 mV/decade for ammonium and urea, respectively as shown in the Table. In constrast to that of Deng et al. (3), the response time was shorter and the slope was higher in our study. This could be attributiced to the difference in urease type and immobilization technique used in this study. The coefficient of variation was found to be 5.06% and 3.20% for ammonium electrode and urea electrode, respectively.

The influence of experimental parameters including pH, buffer composition and immobilization techniques were also determined for optimum analytical performance(3). In this work, the buffer and the optimum pH were chosen as Tris-HCl buffer pH 7.4 to prolong the usefulness of the enzyme electrode according to Neto et al.(12). It has been reported that the concentration of the glutaraldehyde-bean meal conjugate effected the lifetime, response and the activity of the electrode(3).

In our study, we used 15 μL of 2.5% glutaraldehyde which has reported as the optimum amount of glutaraldehyde for enzyme immobilization as above.

Selectivity of the Enzyme Electrode

All the standard urea solutions were prepared without of sodium and potassium. Proceeded with the addition of proper amounts of these compounds in order to simulate the content of such ions in solution. Calibration curves of urea were drawn in order to determine the function of the electrode in the presence of interferences. As it can be seen in Fig. 3 represents a calibration curve of urea with and without sodium and potassium.

The final concentrations of Na^+ and K^+ were 0.05 M. The K_{ij}^{pot} values were calculated by using the following equation (13):

$$K_{ij}^{pot} = C_i \ / \ C_j^{1/n}$$

where C_i and C_j are the concentrations of urea and the interfering substances, respectively. The charge of the foreing cation, n, was assumed to be equal to unity in the case of monoprotonated organic species.

The value of K_{ij}^{pot} was found as $1.4x10^{-3}$ for sodium and potassium ions. At lower concentrations (C_i <10^{-3} M), interference of Na^+ and K^+ appeared to be significant.

It is shown that in the presence of interfereces the linearity range is narrow but still useful for analysis. Our results were in agreement with those of Palleschi et al. (7). Thanei-Wyss et al. (9) reported that their electrodes for NH_4^+ determination did not respond preferentially to any of the most prominent interferring species (K^+, Na^+,Ca^{2+}, Mg^{2+}).

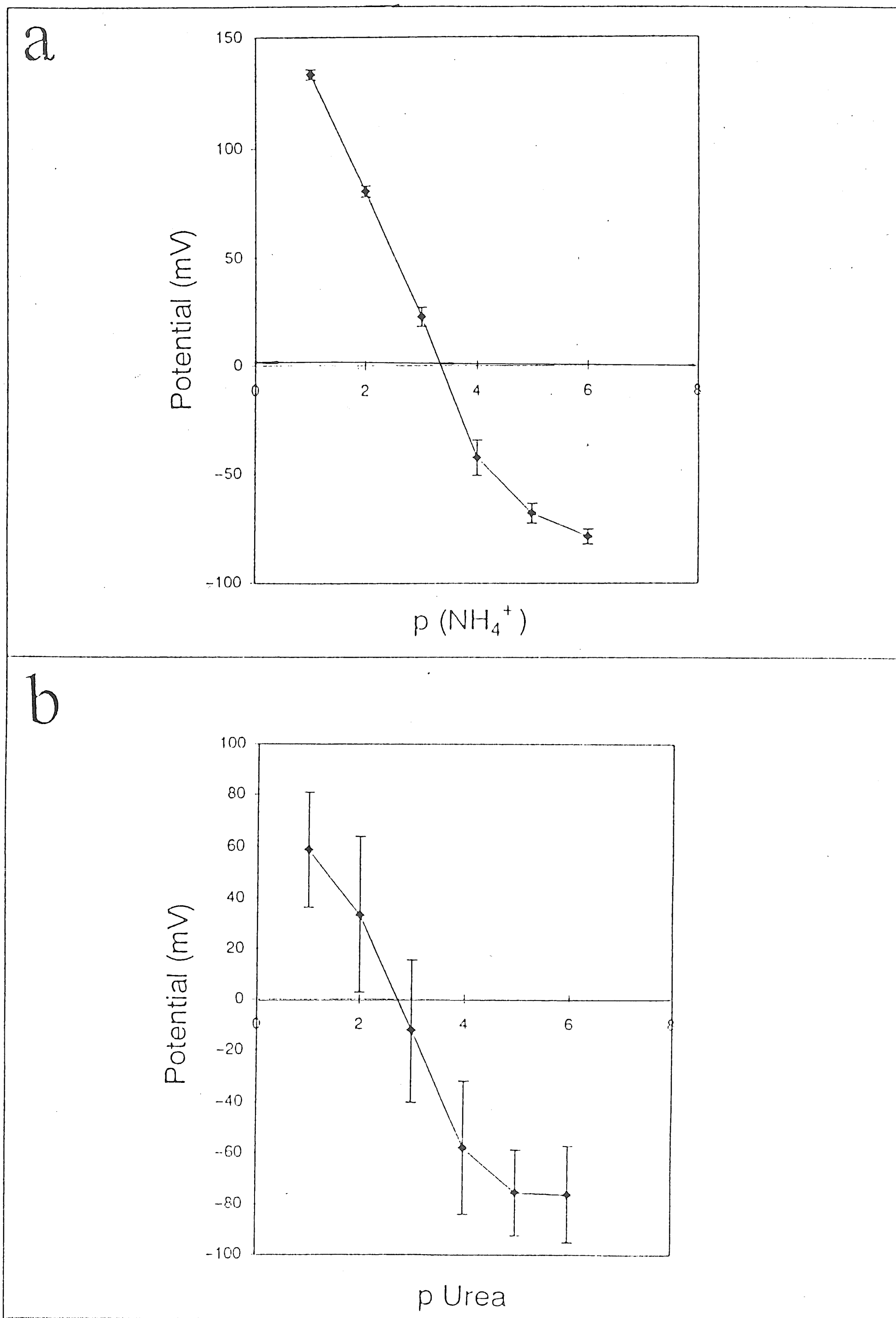


Fig.2. Calibration curve for

a) NH_4^+ with the nonactin based NH_4^+ selective electrode

b) Urea with the enzyme electrode. Other conditions as in Fig. 1.

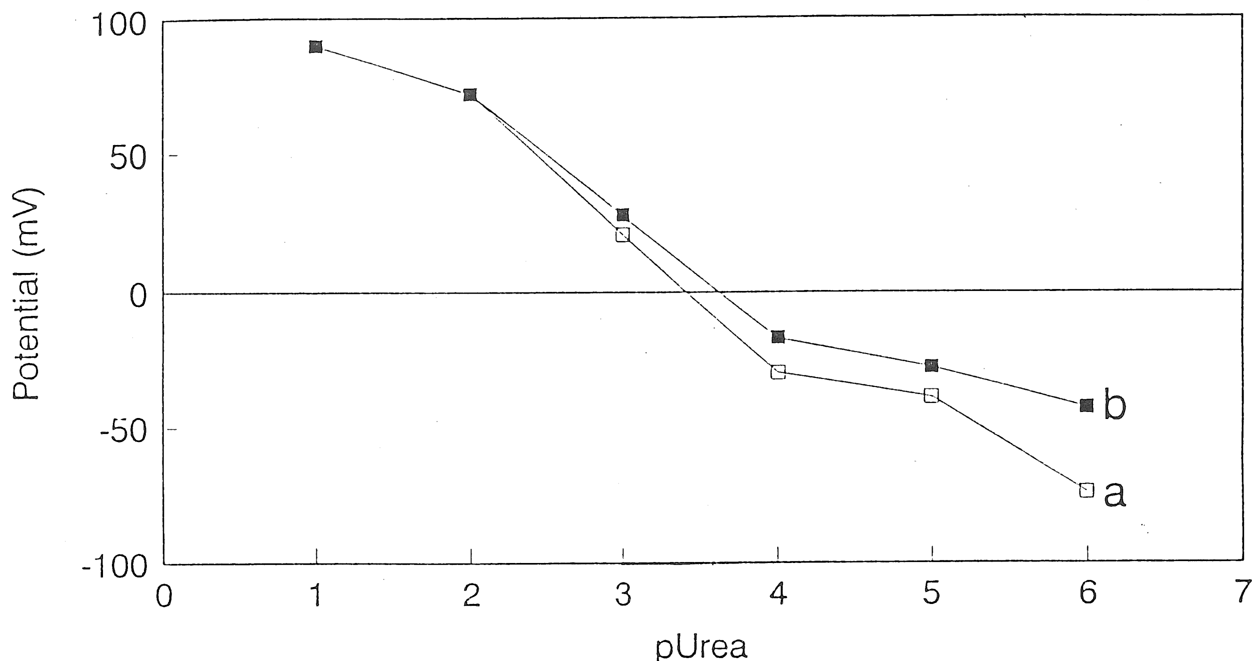


Fig.3. Calibratioin curves of urea in the absence (a) and in the presence (b) of sodium 0.05 M and potassium 0.05 M. Other condition as in Fig. 1.

Palleschi et al.(7) determined urea in human serum with negligible interference, good presicion, and found results in good agreement with those obtained with the clinical standard spectrophotometric method. Shen et al.(8) also used a nonaction-based sensor for NH_4^+ determination in waste water and river water by a differential pH method using flow injection potentiometry. It was obvious that the nonactin based sensor was useful for NH_4^+ determination after enzyme was immobilized.

The proposed potentiometric method for the determination of ammonium using a nonactin based electrode and urea with enzyme electrode after immobilizing Jack bean meal has the advantages of simplicity and rapidity.

In conclusion, the procedure was simple, inexpensive and seemed practical for ammonium and urea determination with good precision in comparison to the other methods.This biosensor is inexpensive and easy to prepare yet analogous measurements of ammonium and urea can be accomplished by using other polymers.

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