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New Urea Biosensor Based on Urease Enzyme Obtained from *Helycobacter pylori*

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Abstract The urease enzyme of *Helicobacter pylori* was isolated from biopsy sample obtained from antrum big curvature cell extracts. A new urea biosensor was prepared by immobilizing urease enzyme isolated from *Helicobacter pylori* on poly(vinylchloride) (PVC) ammonium membrane electrode by using nonactine as an ammonium ionophore. The effect of pH, buffer concentration, and temperature for the biosensor prepared with urease from *H. pylori* were obtained as 6.0, 5 mM, and 25 °C, respectively. We also investigated urease concentration, stirring rate, and enzyme immobilization procedures in response to urea of the enzyme electrode. The linear working range of the biosensor extends from 1×10^{-5} to 1×10^{-2} M and they showed an apparent Nernstian response within this range. Urea enzyme electrodes prepared with urease enzymes obtained from *H. pylori* and *Jack bean* based on PVC membrane ammonium-selective electrode showed very good analytical parameters: high sensitivity, dynamic stability over 2 months with less decrease of sensitivity, response time 1–2 min. The analytical characteristics were investigated and were compared those of the urea biosensor prepared with urease enzyme isolated from *Jack bean* prepared at the same conditions. It was observed that rapid determinations of human serum urea amounts were also made possible with both biosensors.

Keywords *Helicobacter pylori* · Urease enzyme · Isolation · Urea · Polyvinylchloride · Carboxylated polyvinylchloride · Palmitic acid · Nonactin

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Introduction

The spiral Gram-negative microaerophilic bacterium *Helicobacter pylori*, known previously as *Campylobacter pylori*, characterized by significant urease activity, has been implicated as an etiologic agent of gastritis including carcinoma and as a possible contributor to the pathogenesis of peptic ulcer disease [1]. Chronic infection with *H. pylori* results in gastric and duodenal ulcers and is a risk factor for gastric adenocarcinoma. Urease (urea amidohydrolase [EC 3.5.1.5]), produced in abundance by *H. pylori*, is central to the pathogenesis of *H. pylori* infection and disease, as evidenced by the failure of urease-negative mutants to colonize mice and gnotobiotic piglets [2].

In clinical analysis, urea is a very significant parameter as excess in blood serum from its permissible range causes dysfunction of the kidney. Hence, its analysis is significant and is carried out frequently in various laboratories [3]. Various methods used for the determination of urea (without use of biocatalyst) are gas chromatography and calorimetric and flourimetric analysis etc. [4]. However, these methods need sample pretreatment which stands as a major disadvantage in their versatility of applications. Besides, these methods cannot be used for field monitoring. Therefore, devices developed based on biocatalyst "urease" to analyze urea also known as urea biosensors are of vital importance.

Many biosensors have been developed for the determination of urea in the biological samples namely spectrometry [5, 6], potentiometry with application of pH-sensitive electrode or an ion-selective electrode or an ion-sensitive field effective transistor [7, 8], conductometry [9, 10], coulometry [11], amperometry [12], and inductometry [13]. Among these methods, detection through electrochemical mode is highly adaptable and versatile. This method involves the use of electrochemical urea biosensor. In the development of electrochemical urea biosensors, immobilization of urease over electrodes is the key parameter which decides the sensitivity and reproducibility of the sensor [14].

The normal urea range in human serum is between 1.7 and 8.3 mmol/l and levels increase up to 100 mmol/l under pathophysiological conditions [15]. Conventional urea sensors based on pH electrodes [16–20] and ammonium-selective electrodes have been used to detect the hydrogen ions or ammonium ions produced in the enzymatic reaction. Enzymatic sensors for the potentiometric determination of urea belong to the best known class of biosensors. In these biosensors a substrate-active enzyme layer is placed directly on the surface of a classical sensor which measures the concentration of products formed in the course of an enzymatic reaction [4, 21–24].

Urease (EC 3.5.1.5) is an important enzyme in biological systems. Urea is hydrolyzed by urease according to the reaction

$$(NH_2)_2CO + 2H_2O + H^+ \rightarrow 2NH_4^+ + HCO_3^-$$

The potential of ammonium electrode changed the result of changing of ammonium concentration formed after enzymatic reaction is proportional to urea concentration in serum sample. Therefore, ammonium ions produced during enzymatic urea hydrolysis change the potential of the electrode. This changing is proportional to the urea concentration [25–27].

The aim of this work, a new urea biosensor prepared by immobilizing urease enzyme isolated from *H. pylori* was to develop by using carboxylated PVC and PVC ammonium-selective membrane containing palmitic acid (a long-chain fatty acid) and nonactine as an ammonium ionophore to determine urea in serum samples of hemodialysis patients.

Materials and Method

Reagents and Apparatus

Urease (EC 3.5.1.5) was obtained from Merck (Darmstadt, FRG). 1-ethyl-3-(3-dimetylaminopropyl)carbodiimide was obtained from Sigma Chem. Co. (St. Louis, USA). Nonactin, palmitic acid, bis-(2ethyl)hexyl sebacate (DOS), poly(vinylchloride) (PVC), carboxylated poly(vinylchloride)(PVC–COOH) was obtained from Fluka. All other chemicals used were of analytical reagent grade. Standard solutions and buffer solutions were prepared with deionized water.

The human serum samples were provided by Rentek Dialysis Center. The biopsy samples for urase isolation were obtained from Samatya Hospital, Istanbul. Potential and pH measurements were carried out with an ORION 720A model pH-ionmeter.

Isolation of Urease Enzyme from *Helicobacter pylori*

Collection of Biopsy Samples

For biopsy, samples were taken from the antrum big curvature for culture according to the special method because several other bacteria may be the area between the mouth and stomach. First of all, 500 ml of sterile water, 100 ml of 0.8% sterile saline solution (saline), 1.5 ml of sterile ependorf tubes, 20 ml of 70% ethyl alcohol, and sterile needle tip were ready for use. The biopsy tongs and endoscopy device routinely used during 15 min were treated with disinfectants containing 10% formalin by nurses before biopsy was taken. After the disinfection of endoscopy equipment, 50 ml of sterile water was passed from the biopsy channel. In addition, the tip of the biopsy pince was immersed into alcohol and sterile saline, respectively. It was made sure that the first biopsy was taken for culture during the operation. The first biopsy taken from the antrum was put into sterile ependorf tube and two to three drops of sterile saline was added. The tube containing biopsy sample was stored into special cold batteries at 4 °C. There was no serious yield loss when it was kept 4–5 h in this manner.

Isolation of Bacteria from Biopsy Samples

The isolation of bacteria from biopsy samples were only applied to the symptomatic patients. A new method was developed by utilizing conventional methods for the isolation of *H. pylori* from biopsy samples. The biopsy samples stored at 4 °C in a sterile environment (under laminar flow) were placed on sterile glass surfaces by sterile lancets. By this step, it was made sure not to dry the biopsy remains. Biopsy parts were inoculated onto freshly prepared tryptone soy agar with 5% horse blood or Colombia agars with 5% horse blood. The new cultivated medium layers were incubated at incubator containing 10% CO_2 at 37 °C and above 90% humid microaerobic atmosphere for a week. It was showed much attention to maintain the humidity within the incubator. For this purpose, the wet paper towel was put on incubator shelves. After a week, the culture media was checked for bacteria. The colony morphologies of bacteria (white, bright, small colonies), resulted negative to Gram staining, the spiral structures under a microscope, resulted positive to urease, catalase, and oxidase tests show that the bacterium in the medium was *H. pylori.*

The Production and Harvesting of H. pylori

Because of the possibility that different *H. pylori* strains can be found, the inoculation of single colony cultivation on a new medium was carried out. *H. pylori* strains obtained by single colony cultivation were taken in the production. When about 40 layers of bacteria it was already obtained, the production was stopped. Bacterial colonies were transferred into sterile phosphate-buffered saline in medium by using sterile rods and harvested. The colonies collected over agar were combined in 1.5-ml ependorf tube containing buffered saline. About 40 ml of harvest obtained were stored at −70 °C until biosensor preparation. The harvest obtained were taken into the sterile glycerin storage solution, stored at −20 °C by freezing with shock in the half layer of bacteria nitrogen tank for future work.

Urease Enzyme Extraction from Bacteria

The urease enzyme is located within the cell and on the surface of bacteria. In this study, urease enzyme located only the surface of bacteria was extracted. For the enzyme extraction, the bacterial suspensions harvested and stored in a deep freezer were dissolved at room temperature, combined in 50-ml Falcon tube. The collected suspension was centrifuged at 4 °C at 5,000 rpm for 5 min. The süpernatant and pellet were separated and centrifuged again at 4 °C and 5,000 rpm for 90 min. The new supernatant obtained and pellet were separated into another Falcon tube. The purification buffer of 10× (pH 7) was added to the supernatant at the rate of 1:10. Thus, the last solution won the feature of the buffer of 1× (pH 7). The supernatant solution is transferred to 50-ml syringe and 0.20-mm syringe was filtered from filter and sterilized. The enzyme activity and protein content of filtrate were determined by using Berthelot methods [28]. Activity was seen and kept at 4 °C to prepare urea biosensor.

Preparation of Ammonium-Selective Sensors

To prepare ammonium-selective electrodes, ammonium-selective membranes were prepared by using PVC containing palmitic acid and carboxylated PVC are shown in Table 1.

Each membrane compositions in Table 1 was prepared by dissolving about 400 mg membrane components in 5 mL of tetrahydrofurane. The solutions were poured on a glass plate inside a glass ring (42 mm diameter). After 24 h of drying at room temperature, the membrane was formed (approximately 0.5 mm thickness). Disks of 5 mm diameter were

Table 1 The membrane compositions of ammonium-selective electrodes prepared by using PVC containing palmitic acid and carboxylated PVC

Membrane components	Membrane composition (%)	
	A	B
PVC	30.0	–
Palmitic acid	3.0	–
Carboxylated PVC	–	32.5
Nonactin	3.0	3.0
Bis-(2-ethyl)hexyl sebacate (DOS)	64.0	64.5

cut out and attached to the glass electrode body. The inner electrode solution was 1.0×10^{-2} M ammonium chloride (pH 7.0).

Preparation of Potentiometric Urea Biosensors

After determining the sensitivity of ammonium-selective membrane electrodes prepared with each membrane (membranes A and B) against ammonium, urea biosensors were prepared by urease immobilizing urease enzymes obtained from *Jack bean* and *H. pylori* on these electrodes. Enzyme was chemically immobilized on membrane electrode surface according to the same procedures given by [3]. The urea biosensor was prepared by urease solution obtained from *H. pylori* as described below. Fifty microliters of urease solution obtained from *H. pylori* was directly immobilized onto PVC membrane containing palmitic acid.

Others were prepared as follows: 30 mg urease, and 5 mg 1-ethyl-3-(3-dimethyl amino propyl) carbodiimide were dissolved in 1 mL deionized water. Fifty microliters of this solution was dropped on the membrane of ammonium-selective electrode and left overnight, then 50 μL of 2.5% glutaraldehyde solution was deposited on the surface of electrode and the electrode was left for a half hour. Then the electrode surface was washed with deionized water to remove the excess glutaraldehyde. Again 30 mg urease was dissolved in 1 mL deionized water, then 50 μL of this solution was deposited on the electrode surface. The electrode was left overnight. To remove the excess of unbounded enzymes, biosensor was left for 1 h vigorously stirred phosphate buffer (pH 7.0, 10 mM). When not in use, biosensor was stored in a refrigerator at 4 °C.

After separately immobilizing both urease enzymes on two membranes, four different urea biosensors were obtained. These urea biosensors are shown in Table 2.

Measurement of the Biosensor Response to Urea

Potential measurements for urea biosensors were carried out by varying urea concentration in steady-state condition. Five millimolars of TRIS buffer was used as a working buffer solution (pH 7.0). The following electrochemical cell was formed with the proposed creatine biosensors by using a reference electrode:

$$\text{Reference electrode}//\text{urea solution}/\text{membrane}/1.0 \times 10^{-2}\text{M}\,\text{NH}_4\text{Cl}(\text{pH}7.0)/\text{Ag, AgCl}$$

Measurements were made with the proposed urea biosensor and reference electrode. Biosensor was immersed to a depth of 1.5 cm in urea solution and stirred with a magnetic stirrer. The pH values were determined using an Orion combined glass-pH electrode. All the experimental works were carried out at 20±1 °C. The calibration curves were obtained by plotting the potential values of a series of standard urea solution against the logarithm of urea concentration.

Table 2 Urea biosensor by preparing two membrane and urease enzymes

Biosensor name	Membrane	Urease enzyme source
I	A	Commercial *Jack bean*
II	B	Commercial *Jack bean*
III	A	*Helicobacter pylori*
IV	B	*Helicobacter pylori*

Determination of Urea in Serum

Human serum samples were collected from hemodialysis patients at Rentek Dialysis Center. The urea levels in human serum sample was determined by using the standard addition method. The urea biosensor was immersed in the 5 mM TRIS buffer containing certain amount of serum. Then, stock urea solution was added to this solution. Potential values against urea concentrations were plotted and total ammonium amount was determined. Additionally, ammonium concentration in serum samples was determined with standard addition method by using ammonium-selective electrode prepared by the same procedure. Urea concentration in serum was calculated by substracting this value from total ammonium amount [29]. The results were compared to that of obtained from dialysis center.

Validation and Recovery Studies Using Human Serum Samples

To validate the biosensors responses, the results obtained from dialysis center were employed to compare with the biosensors responses. The recovery study involved recoveries of added urea from human serum samples. The urea concentration of each added serum sample was determined by biosensor method. Potential values were also recorded for each serum sample without the addition of urea. The percentage of recovery of urea from all samples for the biosensor method was calculated according to:

$$\%\ \text{Recovery} = \text{Cs}/C \times 100\%$$

Where Cs is the concentration of urea determined by the each biosensor and C is the actual concentration of urea added [30].

Results and Discussion

In this work, carboxylated PVC and PVC matrix containing palmitic acid were used as a membrane matrix and urease enzyme isolated from *H. pylori* was covalently immobilized on this membrane matrix. Until now, carboxylated PVC and PVC matrix containing palmitic acid has been used as a membrane matrix for construction of urea biosensors based on ammonium electrode, and urease has been chemically immobilized on this membrane surface [3, 23, 31–33]. The results of some other urea biosensors reported in literature were presented in Table 3.

Table 3 Some analytical performances of urea biosensor

Electrode	RT	Slope (mV/dec.)	Linear range	Reference
AE, PVC, N	4 min	53	10^{-3}–10^{-6} M	[34]
pH E, PVC-NH_2	2–5 min	50	0.1–30 mM	[35]
AE, P-COOH, N	2–4 min	57	0.3–70 mM	[23]
AE, PVC-NH_2, N	10 s	48	5×10^{-4}–5×10^{-2} M	[36]
GPME, PEF	15–30 s		$1\times10^{-2.5}$–$1\times10^{-1.5}$ M	[37]
PVA–PAA CM	120 s		1–1,000 mM	[38]

AE ammonium electrode, *RT* response time, *PVC* polyvinylchloride, *P-COOH* carboxylated polyvinylchloride, *PA* palmitic acid, *N* nonactin, *pH E* pH electrode, *PVC-NH₂* aminated PVC, *GPME* glass-sealed platinum microdisk electrodes, *PEF* polyethylenimine films, *PVA–PAA CM* PVA–PAA composite matrix

Potentiometric measurements were made by determining ammonium ions produced after enzymatic reaction of urease from *Jack bean* and urease isolated from *H. pylori* in PVC membrane surface of biosensor. Urea is decomposed by urease to electroactive species (ammonium ion) according to the reaction:

$$\text{Urea} + 2H_2O \rightarrow 2NH_4^+ + HCO_3^-$$

The results obtained from urea biosensors prepared with urease enzyme isolated from *H. pylori* and commercial *Jack bean* were compared.

The Sensitivity of Urea Biosensors to Ammonium Ions

In order to understand sensitivity of the urea biosensors to ammonium ions, mV measurements were made with each biosensor in a series of ammonium chloride solutions. Then calibration graphs were plotted and linear working ranges for ammonium ion and slopes of electrodes are given in Table 4.

It can be seen from Table 3 that the sensitivities of all urea biosensors towards ammonium ion are very good and in the range of 1.0×10^{-1}–1.0×10^{-6} M concentration. Therefore we have decided that these urea biosensors can be used for urea determination in biological fluids.

The Sensitivity of Urea Biosensors to Urea

To determine the sensitivity of these biosensors towards urea, a series of urea solutions were prepared and the pH values of these solutions were adjusted to pH 7.0 with trizma-base. The calibration curves were obtained and the working ranges and the slopes for all biosensors were determined from these curves. The linear range of these sensors extends from 1.0×10^{-5} to 1.0×10^{-2} M and they showed an apparent Nernstian response within this range (Fig. 1).

All biosensors were found 1.0×10^{-2}–1.0×10^{-5} M and they have a longer working range than those of most of the reported biosensors. The linear ranges of the urea biosensors were found to be to 7.10^{-2}–3.10^{-4} M by [23], 10^{-3}–10^{-5} M by [34], and 2.1×10^{-2}–7.2×10^{-5} M by [4]. It can be concluded that the biosensors prepared with urease enzymes obtained from *H. pylori* are as advantages as that of prepared with *Jack bean* by using PVC and carboxilated PVC matrix materials for urea determination.

Effect of Buffer Concentration

To examine the effect of buffer concentration on the response of urea biosensors, the slopes of the urea biosensors were measured at six different buffer concentrations varying from 5

Table 4 The linear working range and slopes of urea biosensors for ammonium ions

Urea biosensor	Slope* (mV/p[NH_4^+]) ± s	Linear range
I	40.3±2.4	1.0×10^{-1}–1.0×10^{-6} M
II	35.6±2.3	1.0×10^{-1}–1.0×10^{-6} M
III	33.5±1.5	1.0×10^{-1}–1.0×10^{-6} M
IV	30.4±1.8	1.0×10^{-1}–1.0×10^{-6} M

*Standard deviation

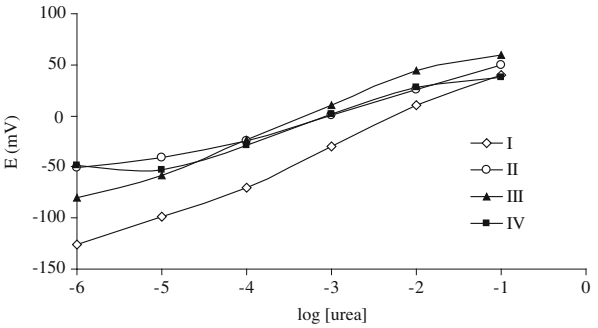

Fig. 1 The calibration graphs of urea biosensors named I, II, III, and IV

to 30 mM. Nernstian responses for all urea biosensors were obtained. The optimum buffer concentrations were determined 5 mM for the biosensor prepared with urease from *H. pylori*, 10 mM for the biosensor prepared with carboxilated PVC membrane, 15 mM for the biosensor prepared with PVC containing palmitic acid membrane (Fig. 2). The reason that the slope (sensitivity) of the biosensors prepared with urease isolated from *H. pylori* were very low at the buffer concentrations higher than 5 mM can be explained that the ionic strength formed by buffer is high and, therefore, it can be prevented reversible binding on membrane of ammonium ion in test solution. Buffer concentrations lower than 5 mM were not investigated due to poor buffering capacity of the solution and the proposed biosensors gave Nernstian responses. Koncki et al. were also described that any increase of the buffer concentration caused a decrease of sensitivity [33]. This is in good agreement with our results. The slopes of III and IV biosensors sharply decreased after 10 and 15 mM, respectively. A decrease of sensitivities of urea biosensors prepared by using biosensors I and II were lower than those of biosensors III and IV as the buffer concentration.

Effect of Buffer pH

Effect of buffer pH on the analytical signal of the urea biosensors was investigated by measurements in TRIS buffer at their optimum pH at six different pH in range of 6.0–8.0. It is shown in Fig. 3 that the slopes and sensitivities of urea biosensors prepared with urease from *H. pylori* are gradually decrease after pH 6.0. Above pH 6.0, urease is denaturated due to deprotonation at active site [3]. The optimum pH of other both urea biosensors prepared with carboxylated PVC membrane and PVC containing palmitic acid membrane were determined as pH 7.2. These results are agreement with the results reported in the literature

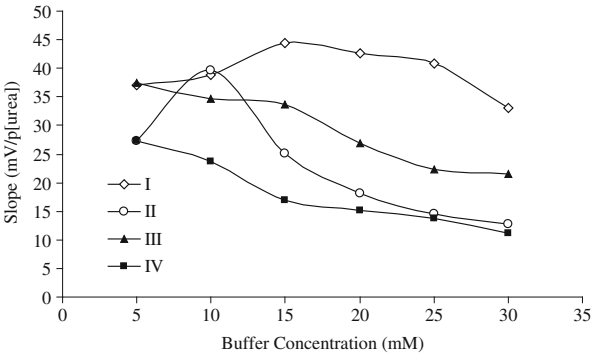

Fig. 2 Effect of buffer concentration on urea biosensors

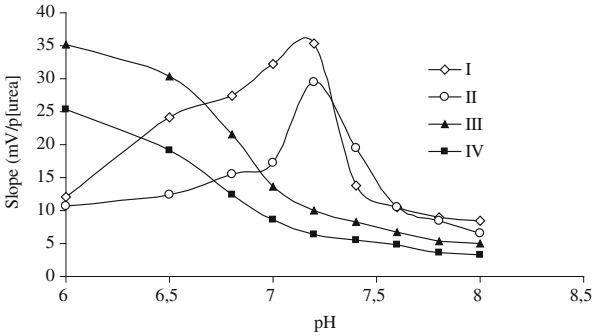

Fig. 3 Effect of pH on urea biosensors

[3, 4, 19, 23, 29, 34]. At pH less than 7.2, urease can be denaturated and hence reduce enzyme activity. Above pH 7.0, urease is denaturated due to deprotonation at active site [12]. The response was also diminished with increase in buffer pH which is common properties of the potentiometric systems [39]. Other measurements of all biosensors were made at their optimum pH.

Effect of Temperature

Effect of temperature on analytical signal of prepared urea biosensors was investigated by measuring for six different temperatures changing from 20 °C to 45 °C. It is shown in Fig. 4 that the optimum temperatures are 30 °C for urea biosensors III and IV and 25 °C for urea biosensors I and II. Therefore, it was thought that urea determinations can be carried out at room temperature by using these sensors. Karbue et al. found the optimum working temperature of the urea biosensor prepared by immobilizing urease with different procedure was also 37 °C [19]. The cause of reduction after optimum temperature of these urea biosensors is because of denaturation of urease or PVC membrane instability at higher temperature.

Selectivity Coefficients of Urea Biosensors

The potential interfering ions such as Na^+, K^+, Ca^{+2} (as chlorides, pH 7.0) that may affect the urea measurement with prepared urea biosensors were tested and their selectivity coefficients were evaluated by the separated solution method according to the IUPAC recommendation [40].

It is shown in Table 5 that the interference effects of Na^+, K^+, Ca^{+2} ions on the response of the urea biosensors were low, but K^+ ions showed the most interference among these

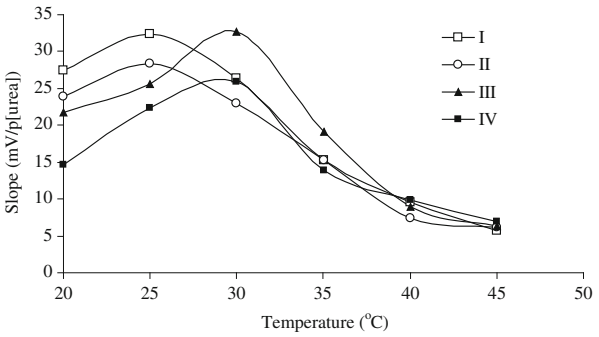

Fig. 4 Effect of temperature urea biosensors

Table 5 The selectivity coefficients of urea biosensors

Ion	Selectivity coefficients, $K_{NH4,X}$			
	Urea biosensors			
	I	II	III	IV
Na^+	1.2×10^{-2}	2.6×10^{-2}	4.7×10^{-2}	9.4×10^{-2}
K^+	1.1×10^{-1}	5.6×10^{-1}	1.5×10^{-1}	1.3×10^{-1}
Ca^{2+}	1.1×10^{-3}	2.5×10^{-3}	4.3×10^{-3}	8.4×10^{-3}

cations. Walcerz et al. determined the selectivity coefficients for their prepared urea biosensors and conclude that K^+ ions showed the most interference effect among other cations [23]. Koncki et al. investigated the interference effect these ions on their prepared biosensor performance and reported that they found no important interference effect of these species [32].

Effect of Stirring Rate

To study the effect of stirring rate on the responses of urea biosensors, the measurements were done in optimum TRIS buffer of each urea biosensor at optimum pH of each urea biosensor at three different stirring rates (100, 250, and 750 rpm) after reaching stable potential. When stirring was made at 100 and 750 rpm levels, the time to reach stable potential was longer than those of 250 rpm rate level. An increase of stirring rate has not resulted to any influence on the magnitude of the analytical signal, but caused an increase in the response time of sensors. This situation can be explained that enzymatic reaction is slow at low stirring rate and probability of enzymatic reaction is decrease because of the changes in the conformation of enzyme at high stirring rate. Therefore 250 rpm is determined by the best stirring rate (Fig. 5).

Response Time

For an ideal biosensor, response time must be short. The values until 5 min are acceptable, but 10 min is deemed to be long. The response time of the urea biosensors was determined by recording the time elapsed to reach a stable potential value after the biosensor and the reference electrode were immersed in calibration solutions. This response time changed between 1 and 2 min. These results were shorter than the results obtained for many urea

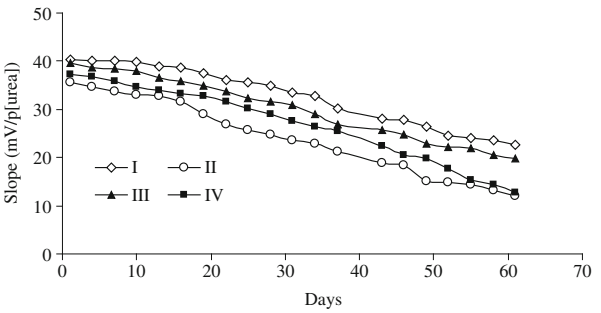

Fig. 5 Storage stability of urea biosensors

Table 6 The relative standard deviations of urea biosensors

	Urea biosensors			
	I	II	III	IV
Relative standard deviation	0.08	0.05	0.2	0.25

biosensors [4, 23, 34]. In conclusion, the response time of the biosensors which we prepared is better than most similar biosensors reported in the literature.

Life Span of the Urea Biosensors

The life span of all urea biosensors were determined by reading the potential values of the calibration solutions and by plotting the calibration curves for a period of 2 months. The slope of the biosensors was observed to show a gradual decrease after 60 days. We can therefore conclude that the lifetime of the proposed urea biosensors is at least 2 months.

Reproducibility of the Urea Biosensors

The biosensors were tested for its reproducibility by plotting the calibration curves for five times within 1 day with same biosensor and the slopes of the biosensors were determined. The relative standard deviation of the slopes was found to be less than 0.5 for all urea biosensors (Table 6). Therefore, we can say that reproducibility of the urea biosensors is high.

Urea Determination in Human Serum Samples and the Validation Results of All Urea Biosensor with Human Serum Samples

Urea amounts in human serum samples of hemodialysis patients obtained from dialysis center were assayed at optimum conditions of each biosensor by using standard addition method. Urea levels in human serum samples obtained by standard addition method were

Table 7 The results of urea content (mg/dL) obtained from the proposed urea biosensors and dialysis center in human serum samples and recovery studies of the biosensors for determining urea

Urea content (mg/dL)									
Urea biosensors									
Sample no.	Dialysis center (C)	I		II		III		IV	
		Cs	Recovery %	Cs	Recovery %	Cs	Recovery %	Cs	Recovery %
1	114	112.7	98.85	108.1	94.82	112.9	99.03	112.8	98.95
2	122	119.5	97.95	111.7	91.56	121.2	99.34	118.9	97.5
3	149	158.2	106.17	136.9	91.88	147.3	98.86	144.9	97.25

Recovery% = Cs/C×100%

correlated with the results obtained from dialysis center. Urea quantities for three different blood samples are given in Table 6.

To evaluate of the sensitivities and demonstrate the analytical applicability of all urea biosensors we prepared, the recoveries of three urea samples were determined by the standard addition method.

Results obtained for the determination of urea in these serum samples using the proposed biosensors and those obtained from dialysis center are in good agreement at 95% confidence level. The results are listed in Table 7. As listed in Table 7, the recovery rate was in the range 91.56–106.17%. There were no significant interferences with other substances in human samples which would have reduced the value of the new urea biosensor-based method. As a result, it can be said that the presented urea biosensors prepared with urease enzyme isolated from *H. pylori* and *Jack bean* based on PVC membrane matrix allowed the determination of urea with comparable accuracy to dialysis center results in human serum.

Conclusion

New types of urea enzyme electrodes prepared with urease enzymes obtained from *H. pylori* and *Jack bean* based on PVC membrane ammonium-selective electrode showed very good analytical parameters: high sensitivity, dynamic stability over 2 months with less decrease of sensitivity, response time 1–2 min. The optimum buffer concentrations were determined 5 mM for the biosensor prepared with urease from *H. pylori*, 10 mM for the biosensor prepared with carboxylated PVC membrane, 15 mM for the biosensor prepared with PVC containing palmitic acid membrane. The slopes and sensitivities of urea biosensors prepared with urease from *H. pylori* are gradually decrease after pH 6.0 because urease is denaturated due to deprotonation at active sites above pH 6.0. The optimum temperatures are 30 °C for urea biosensors III and IV and 25 °C for urea biosensors I and II. We can carry out urea determinations at room temperature by using these sensors. The interference effects of Na^+, K^+, and Ca^{+2} ions on the response of the urea biosensors were low, but K^+ ions showed the most interference among these cations. It was observed that rapid determinations of serum urea concentrations were also made possible.

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