AMPEROMETRIC CARBON PASTE ENZYME ELECTRODES FOR URIC ACID DETERMINATION WITH DIFFERENT MEDIATORS

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In this study, two new amperometric carbon paste enzyme electrodes for determination of uric acid were developed. The carbon paste was prepared by mixing uricase enzyme, 1,4-benzoquinone or poly(vinylferrocene) (PVF) as a mediator, graphite powder, paraffin oil and then the paste was placed into cavity of a teflon electrode body. Determination of uric acid was performed by oxidation of enzymatically generated H_2O_2. The effects of enzyme loading, mediator amount, buffer type, pH, buffer concentration, working potential and temperature were investigated for both electrodes. The working range of the 1,4-benzo-quinone modified enzyme electrode was 1.9×10^{-8}–2.7×10^{-3} M, detection limit 1.9×10^{-8} M and response time 150 s. Optimum buffer type, pH, buffer concentration, working potential, temperature and amounts of enzyme and mediator for 1,4-benzoquinone modified enzyme electrode were found to be Tris, 8.0, 0.20 M, +0.25 V, 30 °C, 2.0 Unit and 13%, respectively. The working range of the PVF modified enzyme electrode was 7.4×10^{-8}–7.0×10^{-3} M, detection limit 7.4×10^{-8} M and response time 120 s. Optimum buffer type, pH, buffer concentra-tion, working potential, temperature and amounts of enzyme and mediator for PVF modified enzyme electrode were found to be phosphate, 8.0, 0.05 M, +0.70 and +0.30 V, 40 °C, 2.0 Unit and 10.9%, respectively. The repeatability, storage stability of the enzyme electrodes and interference effects were also investigated. Enzyme electrodes were used for determination of uric acid in serum samples and the results were in a good agreement with those obtained by commercial enzymatic kits.
Keywords: Amperometry; 1,4-Benzoquinone; Carbon paste; Enzyme electrode; Poly(vinyl-ferrocene); Uric acid; Biosensors; Carbon; Enzymes.

Uric acid is an end product from purine derivatives in human metabolism. The normal level of uric acid in serum is between 240 and 520 μM and 1.4 and 4.4 mM in urinary excretion[1]. Abnormal uric acid level in biological fluids is a marker of several disorders such as gout, renal disease, hyper-uricemia and Lesch–Nyhan syndrome[2]. Many studies have suggested that increase in the serum uric acid level is a risk factor for cardiovascular dis-ease[3] and uric acid level is associated with hypertension[4]. Uric acid also acts

as an antioxidant in human body[5]. Consequently, fast and reliable determination of uric acid in biological fluids is routinely required for diagnosis and treatment.

For uric acid determination, various techniques such as chemiluminescence[6,7], fluorescence[8], HPLC-mass spectrometry[9], capillary electrophoresis-amperometry[10], electrochemistry[11,12], colorimetry[13] and enzymatic test-kits have been reported. However, these methods are usually laborious, expensive, time-consuming and/or complex to perform. Among these techniques, the enzymatic-colorimetric method using uricase and peroxidase together is widely used in routine analysis due to its simplicity, sensitivity and specificity. Test kits of this method are commercially available. However, the cost of uricase and peroxidase used in the kit is a factor that limits widespread use of the method for large number of samples[13].

An alternative method for uric acid determination is the use of electrochemical enzyme electrodes, which allow direct, rapid and inexpensive measurement of uric acid in samples. Various types of enzyme electrodes have been reported for uric acid determination[2,4,14–19].

Uricase (UOX) specifically catalyses the oxidation of uric acid according to the reaction

$$\text{Uric acid} + 2\ H_2O + O_2 \xrightarrow{\text{UOX}} \text{Allantoin} + H_2O_2 + CO_2\,.$$

Enzyme electrodes for uric acid determination is based on the determination of enzymatically generated CO_2 or H_2O_2 or the consumption of O_2 during the enzymatic reaction[15,18,20,21].

H_2O_2 formation can be detected amperometrically during oxidation at the electrode surface[16]

$$H_2O_2 \rightarrow O_2 + 2\ H^+ + 2\ e^-\,.$$
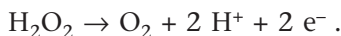
The most important drawback of the uric acid biosensors is the possible oxidation of uric acid itself at the electrode surface such as Pt, Au and carbon at high potentials, which the electrochemical oxidation of H_2O_2 occurs[22,23]. This problem can be solved by the dilution of the sample[24]. At high potentials, other electroactive species present in the sample can also be oxidized and interfere in the analysis[4]. To decrease the working potential and thus the effect of interferences, the use of mediators have been reported for electrochemical oxidation of H_2O_2 in uric acid biosensors[17,25]. Another alternative is to use horseradish peroxidase enzyme (HRP) together with uricase.

This leads to the enzymatic reduction of H_2O_2 at lower potentials[22] which decreases interference effects.

Carbon paste electrodes have been widely used for electroanalytical applications since their introduction by Adams in 1958 due to their advantages such as low background current, low cost of fabrication and ease of modification[26]. A number of uric acid biosensors based on carbon paste matrix have been reported[2,21,22]. The use of redox mediators in carbon paste electrodes is a promising approach to modified electrodes[27,28].

In this work, we developed simple and low cost enzyme electrodes working at low potentials to eliminate the effects of interferences and perform reliable uric acid analysis in real samples. We constructed two different modified carbon paste enzyme electrodes by the incorporation of uricase and redox mediator poly(vinylferrocene) or 1,4-benzoquinone within a carbon paste matrix. We investigated the parameters that influence the electrode performance, the analytical characteristics, operational and storage stability and the effect of interferences. We applied the developed enzyme electrodes for uric acid determination in real samples.

EXPERIMENTAL

Equipment and Reagents

The electrochemical studies were carried out using IVIUM electrochemical analyzer (Ivium Technologies, Netherlands) using a three-electrode cell stand (Bioanalytical Systems, Inc., USA). The working electrode was a modified carbon paste electrode. The counter and reference electrodes were a Pt wire (BAS MW 1034) and Ag|AgCl electrode (BAS MF 2052) (Bioanalytical Systems, Inc., USA), respectively. The pH values of the buffer solutions were measured with ORION Model 720A pH/ion meter and ORION combined pH electrode (Thermo Scientific, USA). Temperature control was achieved with Grant LTD GG thermostat (Grant Instruments, UK).

Uricase (E.C.3.5.3.3. from *Arthrobacter globiformis* sp. with a specific activity of 18 Units/mg solid), uric acid, ascorbic acid, methionine, urea and glutaraldehyte were purchased from Sigma (St. Louis, MO, USA). Sodium monohydrogenphosphate and sodium dihydrogenphosphate were supplied from Riedel–de Haën (Seelze, Germany). 1,4-Benzoquinone, bovine serum albumin (BSA), graphite powder, paraffin oil, glucose, creatinine and tris(hydroxymethyl)aminomethane were from Fluka (Buchs, Switzerland). Vinylferrocene and aspartic acid were from Aldrich (Steinheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). Standard solution of uric acid was prepared by dissolving uric acid in 4% Li_2CO_3 aqueous solution[18]. The standard uric acid solutions were prepared freshly every day and immeaditely wrapped with aluminium foil to prevent biomolecule degradation. PVF was prepared by the chemical polymerization of vinylferrocene[29]. All measurements except temperature study were carried out at room temperature (23 ± 2 °C).

Preparation of Carbon Paste, 1,4-Benzoquinone or PVF Modified Carbon Paste and
Carbon Paste Enzyme Electrodes

For unmodified electrode construction carbon paste was prepared in the following propor-
tions: 65.2% graphite powder and 34.8% paraffin oil. 1,4-Benzoquinone modified carbon
paste electrode (B-MCPE) was composed of 52.2% graphite powder, 13% 1,4-benzoquinone
and 34.8% paraffin oil. PVF modified carbon paste (PVF-MCPE) was composed of 54.3%
graphite powder, 10.9% PVF and 34.8% paraffin oil. The modified electrodes were prepared
by hand-mixing graphite powder with the mediator and then paraffin oil was added and
throughly mixed for approximately 20 min to form the homogeneous modified carbon
paste electrodes.

For 1,4-benzoquinone modified enzyme electrode (B-MCPEE) and PVF modified enzyme
electrode (PVF-MCPEE) graphite powder and mediator were mixed and enzyme solution (50
μl uricase (40 Unit/ml), 1.5 mg BSA and 10 μl 1.25% glutaraldehyde) was added. After the
evaporation of water paraffin oil was added and mixed for approximately 20 min until a
uniform paste was obtained. In all cases, the paste was then placed into the bottom of the
working electrode body (BAS MP 5023) and the electrode surface was polished with a weight
paper to have a smooth surface. Calibration graphs were plotted for each electrode and
the optimum composition was found by comparing their sensitivities and working ranges.
The electrodes were only washed with distilled water and working buffer between mea-
surements. Electrodes were stored in refrigerator at +4 °C in when not in use.

Amperometric Measurements

All amperometric measurements with 1,4-benzoquinone modified electrodes were performed
in Tris buffer solution (0.20 M, pH 8.0). At first, we investigated the electrochemical oxida-
tion of H_2O_2 at unmodified carbon paste electrodes (CPE). 5.0 ml of Tris buffer solution was
added to the cell. After the application of +0.25 V potential, the background current was al-
lowed to decay to constant value. Then an aliquot of 1×10^{-2} M H_2O_2 stock solution was
added to the cell and the solution was purged with argon and stirred prior to each measure-
ment. The response of the electrode to H_2O_2 was measured after 40 s. The current values
versus H_2O_2 concentrations were plotted in order to determine whether the electrode was
sensitive to H_2O_2. The same experiment was performed with B-MCPE. Amperometric mea-
surements with PVF modified electrodes were performed in phosphate buffer solutions at
+0.70 and +0.30 V (0.05 M, pH 8.0).

The response of B-MCPEE to uric acid was determined at +0.25 V vs Ag|AgCl in Tris buffer
(0.20 M, pH 8.0). The same experiment was performed for PVF-MCPEE at +0.70 and +0.30 V
vs Ag|AgCl.

RESULTS AND DISCUSSION

In this study, we prepared two new carbon paste-based uric acid enzyme
electrodes by using 1,4-benzoquinone or PVF as mediator. Optimum work-
ing conditions, performance factors of the electrodes and effect of interfer-
ences were investigated and discussed below. Real sample measurements
were also performed.

H_2O_2 Responses of Carbon Paste and Modified Carbon Paste Electrodes

We investigated the electrochemical oxidation of H_2O_2 at unmodified, 1,4-benzoquinone modified and poly(vinylferrocene) modified carbon paste electrodes. H_2O_2 sensitivity of the 1,4-benzoquinone modified electrode (0.45 µA mM^{-1}) was found to be much higher than that of unmodified carbon paste electrode (6×10^{-4} µA mM^{-1}) at +0.25 V. H_2O_2 sensitivity of the PVF modified electrode (0.68 µA mM^{-1}) was also higher than that of unmodified carbon paste electrode (7×10^{-2} µA mM^{-1}) at +0.70 V. The sensitivity of PVF-MCPE was higher than the unmodified electrode at +0.30 V, too. It can be concluded that both 1,4-benzoquinone (Q) and PVF$^+$ catalyzes the electrooxidation of H_2O_2. 1,4-Benzoquinone reduces to hydroquinone (H_2Q) and PVF$^+$ to PVF. Hydroquinone at +0.25 V and PVF at +0.70 and +0.30 V are also electrooxidized on carbon paste electrode surface and the oxidized forms, 1,4-benzoquinone and PVF$^+$ are re-formed.

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2\ e^-$$

$$H_2O_2 + Q \rightarrow O_2 + H_2Q$$

$$H_2O_2 + 2PVF^+ \rightarrow O_2 + 2H^+ + 2PVF$$
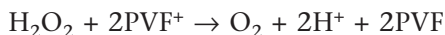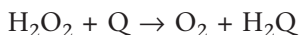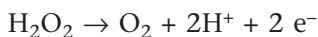
Our results are found to be in good agreement with the data reported in the literature[27,30]. This catalytic processes promote an increase in the sensitivities of the 1,4-benzoquinone and PVF modified carbon paste electrodes. In conclusion these mediators can be used to construct modified enzyme electrodes for uric acid determination.

Optimum Working Conditions and Electrode Compositions of B-MCPEE and PVF-MCPEE

Enzyme and Mediator Amount

The amperometric response of an enzyme electrode greatly depends on the amount of the enzyme loaded. Thus, the effect of the enzyme amount in carbon paste matrix on the response was determined. The responses of the enzyme electrodes were measured at four different enzyme amounts by keeping the amount of mediator constant and varying the uricase amount. Gradual increase in the uric acid sensitivity was observed when the amount of uricase was increased. The maximum sensitivity was observed at the loading of 2 Unit uricase for both of the electrodes. The linearity and the

working range of the calibration graphs plotted for electrodes containing 0.8, 1.2 and 1.6 Unit enzyme were not satisfactory. The increase in the uricase amount resulted in the increase in the active site of the electrode thus the sensitivity of B-MCPEE and PVF-MCPEE.

A study was carried out to assess the effect of 1,4-benzoquinone and PVF amount in carbon paste matrix on the electrode response. Mediator amount varied as 6.5, 8.7, 10.9 and 13%, while enzyme amount was kept constant. The highest sensitivity and working range was obtained with the carbon paste electrodes prepared with 13% 1,4-benzoquinone and 10.9% PVF. As the mediator amount increases the reaction between uric acid and 1,4-benzoquinone or PVF favours the production of hydroquinone or PVF$^+$ and thus an increase in sensitivity is expected.

The Effect of Buffer Type

Tris and phosphate buffers were investigated for the performance of B-MCPEE and Tris, phosphate and borate buffers were investigated for the performance of PVF-MCPEE. The sensitiviy of the B-MCPEE is higher in Tris buffer and the sensitiviy of the PVF-MCPEE is higher with phosphate buffer. Tris buffer was used for B-MCPEE and phosphate buffer was used for PVF-MCPEE for all of the experiments. Beside Tris buffer[2,19,20,31], phosphate buffer[5,14,15,17,21,24], borate buffer[23,32], glycine buffer[33] and BR buffer[16,34] were also reported for uric acid enzyme electrodes.

The Effect of pH

The pH of the working medium affects the enzyme electrode response since ionizing groups control the activity of the enzyme. The effect of pH on the response of B-MCPEE and PVF-MCPEE was investigated at various pH values. The highest sensitivity was obtained at pH 8.0 as can be seen in Fig. 1. Therefore, pH 8.0 was selected as optimum pH for both electrodes and all the following measurements were performed at this pH. Although the reported optimum pH of uricase is in the range of 8.5–9.2[22,35], pH of the most physiological fluids are below this range thus it is suitable to work at a pH of 8.0. The pH study also indicates that immobilization procedure has a little influence on the properties of uricase. The selected pH is in good agreement with the data reported in the literature[20,23,36]. For uric acid enzyme electrodes, different pH values such as 6.5[37], 6.6[22], 6.8[14], 6.9[5], 7.0[21,34], 8.5[4,19], 8.6[2,18] and 9.6[16] were also reported in the literature. This was attributed to the fact that the mediator used, enzyme supply, immobili-

zation method and electrode preparation procedures were different. The decrease in the response of the enzyme electrode at pH values below and above 8.0 can be attributed to the change of the enzyme conformations leading to a decrease in the enzyme activities.

The Effect of Buffer Concentration

The amperometric response of B-MCPEE was determined at different Tris concentrations of 0.05, 0.10, 0.15 and 0.20 M and the best response for B-MCPEE was obtained at 0.2 M. Above or below this concentration, the response was found to show a significant decrease. The reason for this could be the buffering capacity of the solution decreases at lower Tris concentrations resulting in the change of the buffer pH. At higher concentrations, surface of the enzyme electrode is damaged and some of the carbon paste washes out. The amperometric response of PVF-MCPEE was also determined in 0.05, 0.10, 0.15 and 0.20 M phosphate buffer and best response was obtained at 0.05 M.

The Effect of Temperature

Temperature has a great effect on enzyme activity and it is important to investigate the temperature dependence of the response of the enzyme elec-
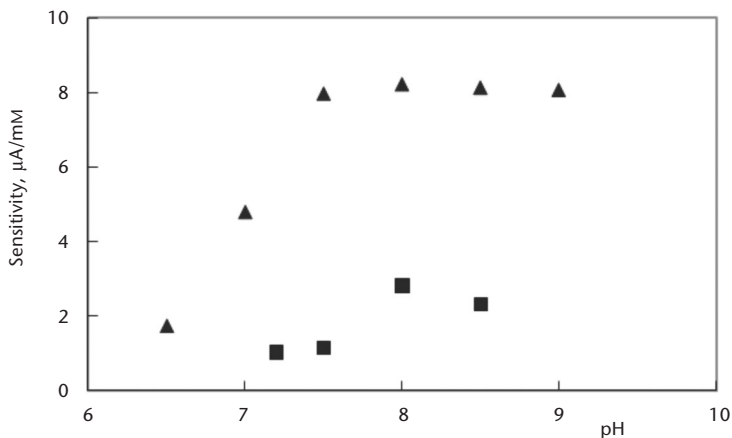

Fig. 1
The effect of buffer pH on the response of the electrodes: ■ B-MCPEE 0.2 M Tris buffer solution, +0.25 V; ▲ PVF-MCPEE 0.05 M phosphate buffer solution, +0.70 V; room temperature

trode. The temperature influence on the responses of B-MCPEE and PVF-MCPEE was tested between 25 and 40 °C at pH 8.0. The response of B-MCPEE increased with temperature up to 30 °C and decreased suddenly afterwards. The sudden decrease after 30 °C is thought to be caused by the denaturation of the enzyme[23]. We performed the uric acid measurements at room temperature (23 ± 2 °C) since the linearity of the calibration curves plotted at 25 °C was better than the linearity of the calibration curves plotted at higher degrees. Working at room temperature also simplifies the experimental procedure and prolongs the lifetime of the enzyme electrode. Although different temperatures like 37 [21,31], 35 [15] and 30 °C [18,38] were reported as optimum, usually uric acid enzyme electrode studies were performed at room temperature[4,14,17,23,39] due to easier working conditions. The response of PVF-MCPEE increased with temperature up to 40 °C. However, we performed the uric acid measurements at room temperature with PVF- MCPEE like B-MCPEE for convenience.

The Effect of Working Potential

The amperometric responses of B-MCPEE and PVF-MCPEE were determined at different working potentials between 0 and +0.70 V. In the +0.25 to +0.60 V range, sensitivity increased for B-MCPEE (data not shown). Although the best sensivity was obtained at +0.60 V, +0.25 V was selected as working potential. Working at low potentials is important to minimize the interference effect when analyzing real samples. This low potential is also important to prevent the direct oxidation of uric acid[2,14,21,22]. +0.70 V was selected as the working potential for PVF-MCPEE. However, we obtained calibration curves with good linearity at +0.30 V with lower sensitivity for PVF-MCPEE. Although we performed all the optimization studies and real sample analysis at +0.70 V, uric acid determination in real samples was also performed at +0.30 V for PVF-MCPEE to overcome the possible interferences of other oxidizable species. For PVF modified biosensors, studies at +0.70, +0.60 and +0.50 V potentials were reported[30,40,41]. In this study, it was shown that +0.30 V can be used for PVF modified enzyme electrodes thus minimizing the interferences in real samples.

Performance Parameters of B-MCPEE and PVF-MCPEE

Response Time

The response time of the enzyme electrode depends on the uric acid concentration thus the amperometric response times of B-MCPEE and PVF-MCPEE to uric acid were determined at two different uric acid concentrations. The current differences for 1.0×10^{-5} and 1.0×10^{-4} M uric acid versus time were plotted. The response time was shorter at lower concentrations than that at higher concentrations. The response time was found to be 150 s (t_{95}) for B-MCPEE and 120 s (t_{95}) for PVF-MCPEE. Since the response curves at high and low concentrations are parallel to each other, the measurements can be taken before 150 and 120 s provided that they are made exactly at the same times. The response times were expected as 40 s and all the parameters were investigated basing upon the measurements taken after 40 s. These response times are quite fast and highly suitable for biosensor response. There are longer: 2 min [25], 330 s [23], 3.5 min [35] and shorter response times 37 [22], 70 [2], 5 [34] and 60 s [37] were reported for uric acid enzyme electrodes.

Repeatability

The repeatability of B-MCPEE and PVF-MCPEE was also investigated. Five calibration curves were plotted by the use of the same electrodes sequentially. The relative standard deviation of the sensitivities (the slopes of the curves) was 4.7% for B-MCPEE and 6.2% for PVF-MCPEE.

Effect of Analyte Concentration

Figure 2 shows the amperometric response of the B-MCPEE recorded as a function of uric acid concentration under optimum conditions. The response current increases with the concentration of uric acid up to 2.7×10^{-3} M. The limit of detection of the enzyme electrode is 5.0×10^{-6} M. The curve is composed of three linear parts ranging from 4.8×10^{-6} to 2.8×10^{-5} M, from 3.5×10^{-5} to 4.9×10^{-4} M and from 6.3×10^{-4} to 2.7×10^{-3} M. This range covers the normal serum uric acid level of 1.3×10^{-4}–4.6×10^{-4} M [1] so uric acid analysis in human serum can be performed by this electrode quite efficiently.

We also investigated the response of the B-MCPEE at lower concentrations and the enzyme electrode showed linear response between 1.9×10^{-8}–

2.8×10^{-7} M (Fig. 3). This response at lower concentrations is important when working with diluted samples to eliminate the interference effects.

The relationship between uric acid concentration and response current of the PVF-MCPEE is shown in Fig. 4. There are two linear parts ranging from

Fig. 2
The effect of uric acid concentration on the response of B-MCPEE (0.20 M, pH 8.0 Tris buffer, +0.25 V, room temperature)

y (μA) = 3.815x (μM) + 0.694
R^2 = 0.9702

Fig. 3
The effect of uric acid concentration on the response of B-MCPEE (0.20 M, pH 8.0 Tris buffer, +0.25 V, room temperature)

5.0×10^{-6} to 9.0×10^{-5} M and from 3.0×10^{-4} to 7.0×10^{-3} M . The limit of detection is 4.0×10^{-6} M.

The PVF-MCPEE showed linear response between 7.4×10^{-8}–2.8×10^{-7} M at +0.70 V (data not shown). Linear working range and limit of detection
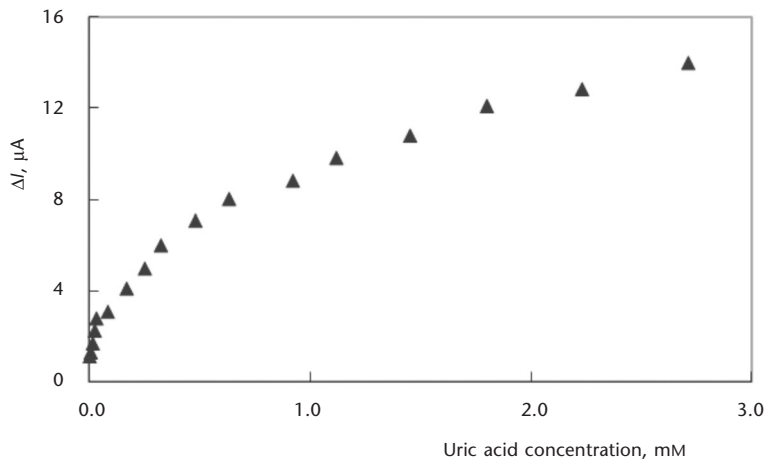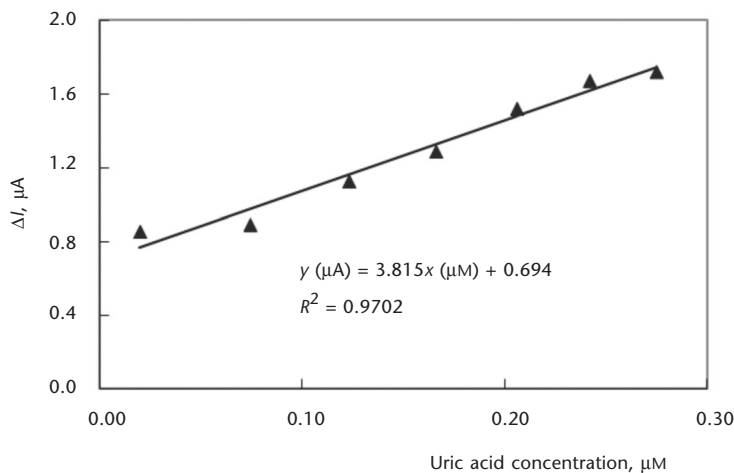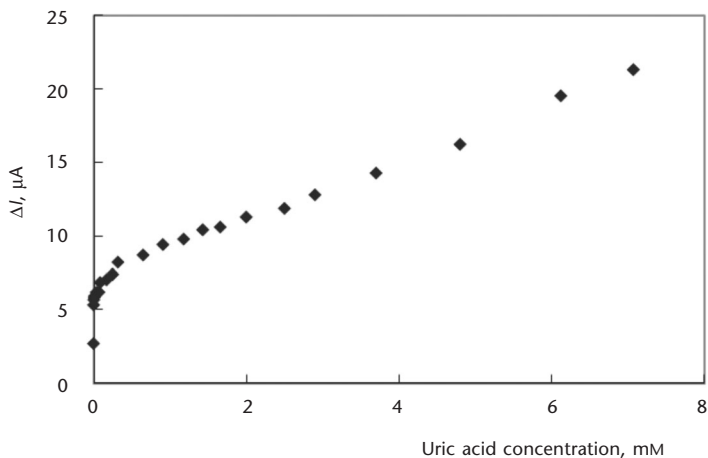

FIG. 4
The effect of uric acid concentration on the response of PVF-MCPEE (0.05 M, pH 8.0 phosphate buffer, +0.70 V, room temperature)
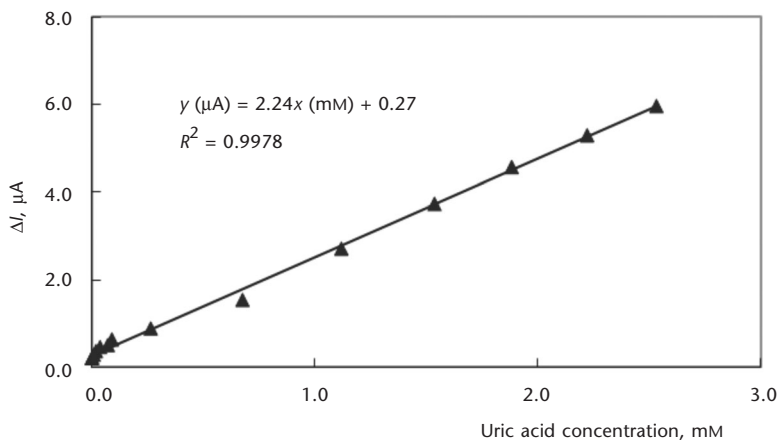

FIG. 5
The effect of uric acid concentration on the response of PVF-MCPEE (0.05 M, pH 8.0 phosphate buffer, +0.30 V, room temperature)

was also determined at +0.30 V for PVF-MCPEE (Fig. 5). Detection limit was 1.0×10^{-6} M and linear working range was 1.0×10^{-6}–2.5×10^{-3} M at +0.30 V.

Storage Stability

We checked the long-term stability of B-MCPEE and PVF-MCPEE prepared under optimum conditions. The electrodes were stored under dry conditions at +4 °C when not in use. Calibration curves were plotted for these electrodes at different days during the storage period. The B-MCPEE lost 37% of its initial sensitivity after 15 days. PVF-MCPEE lost 39% of its initial activity after 7 days, 53% after 14 days and 68% after 29 days. PVF-MCPEE loses 39% of its initial activity after 7 days but B-MCPEE loses 37% of its initial activity after 15 days so it can be concluded that storage stability of B-MCPEE is better than PVF modified one.

Effect of Interferences

The oxidazable compounds such as ascorbic acid, urea, glucose, creatinine and ascorbic acid can interefere with amperometric measurement of enzyme electrodes. In our study the evaluation of interferences was carried out for several chemical species normally present in human serum and urine. Interference effect was determined for both B-MCPEE and PVF-
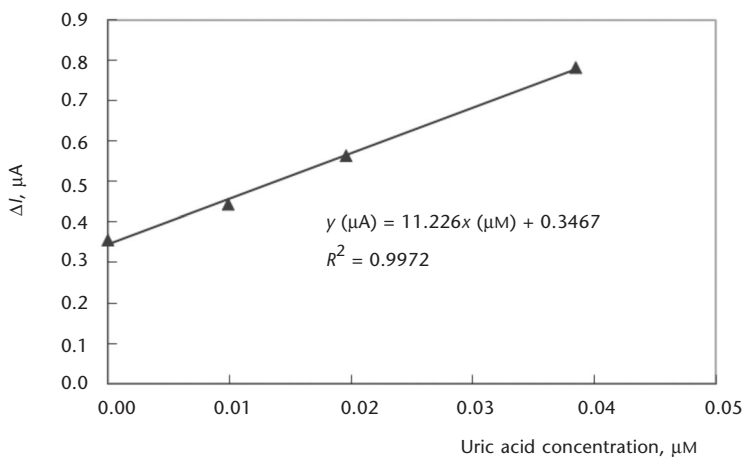

Fig. 6
The standart addition graph for 1.70 mg/dl uric acid with B-MCPEE (0.20 M, pH 8.0 Tris buffer, +0.25 V, room temperature)

MCPEE. Effect of ascorbic acid, glucose, urea, creatinine, methionine and aspartic acid on the response current was investigated at a constant uric acid concentration of 2.0×10^{-5} M. Different concentrations of interfering compounds were chosen. One is to reflect the actual physiological concentration, the other is at a lower concentration to observe the effect in diluted samples. The results of interference study are shown in Table I. Ascorbic acid causes 14.7 and 8.9% interference at the highest concentrations found in urine and serum respectively. However when concentration of ascorbic acid is decreased the interference reduces. This is valid for all species investigated with both of the modified electrodes so we can conclude that dilution reduces the effect of interferences like it was reported in the literature[23,24].

TABLE I
Effect of interferences on the response of modified electrodes

Interfering species	Concentration of the interference, M	Interference % (B-MCPEE)	Interference % (PVF-MCPEE)
Ascorbic acid	3×10^{-4}	14.7	20.6
	1×10^{-4}	8.9	5.6
	1×10^{-5}	–6.5	0.5
Glucose	4×10^{-3}	–17.9	–27.6
	1×10^{-4}	–1.4	–9.7
	2×10^{-6}	–	–0.43
Creatinine	8×10^{-4}	–3.3	–6.1
	1×10^{-4}	–1.7	–2.3
Urea	5×10^{-2}	–29.1	–27.3
	8×10^{-4}	–9.6	–12.6
	4×10^{-5}	–3.5	2.3
Methionine	4×10^{-5}	–9.5	–24.6
	5×10^{-8}	–4.8	1.6
Aspartic acid	5×10^{-3}	–10.5	–19.7
	1×10^{-5}	–1.9	–8.4
	2×10^{-6}	–	–4.9

Determination of Uric Acid in Serum

The proposed B-MCPEE and PVF-MCPEE were used to determine the uric acid in human serum samples. Serum samples were mixed with 10% trichloroacetic acid (TCA) for deproteinization. After a 10-min centrifugation at 3000 rpm, supernatants were filtered and diluted. The modified carbon paste enzyme electrode was placed in the cell containing 5 ml buffer. After the stabilization of background current, an aliquot of sample was added. The solution was purged and stirred then the response of the electrode was measured. After the current reached a steady-state value, standard addition method was used to determine uric acid concentration (Fig. 6). In Table II, results obtained for three serum samples using our new enzyme electrodes are presented together with those obtained from the enzymatic kit. The results are in a good agreement and show that the new enzyme electrodes can be used for uric acid determination in serum samples. We checked the accuracy of the method by t-test. The *t* value is 0.78 for B-MCPEE and 0.73 for PVF-MCPEE at 95% confidence level, for which t_{critic} is 4.30. It can be concluded that there is no difference between the results of two methods at a confidence level of 95%.

TABLE II
Comparison of uric acid content in serum samples using the modified enzyme electrodes and enzymatic kit

	Uric acid (mg/100 ml)		
	B-MCPEE[a]	PVF-MCPEE[a]	Enzymatic kit
Sample 1	1.99 ± 0.40 (+0.25 V)	1.86 ± 0.45 (+0.70 V)	1.70
Sample 2	3.59 ± 0.11 (+0.25 V)	3.43 ± 0.32 (+0.70 V) 3.37 ± 0.13 (+0.30 V)	3.50
Sample 3	4.89 ± 0.30 (+0.25 V)	4.47 ± 0.11 (+0.70 V)	5.00

[a] Results are the mean value of three measurements.

CONCLUSION

In this study, determination of uric acid in serum samples was carried out using 1,4-benzoquinone modified and poly(vinylferrocene) modified carbon paste uric acid enzyme electrodes. The properties and optimum working conditions of the carbon paste enzyme electrodes are summarized in Tables III and IV. It can be concluded that both 1,4-benzoquinone and

poly(vinylferrocene) mediators served quite well to prepare mediated amperometric enzyme electrodes for reliable determination of uric acid in human serum samples. 1,4-benzoquinone allowed the determination of uric acid at a low potential (+0.25 V) hence reducing the interference effect. Uric acid determination was achieved at both high (+0.70 V) and low (+0.30 V) potentials by using PVF. In this study it was shown that effect of

TABLE III
The properties and the optimum working conditions of the enzyme electrodes

Electrode composition			Optimum working conditions		
	B-MCPEE	PVF-MCPEE		B-MCPEE	PVF-MCPEE
Mediator amount	3.0 mg	2.5 mg	Buffer	tris	phosphate
Graphite amount	12 mg	12.5 mg	pH	8.0	8.0
Paraffin oil	10 µl	10 µl	Buffer concentration	0.20 M	0.05 M
Enzyme amount	2.0 U	2.0 U	Temperature	30 °C	40 °C
BSA	1.5 mg	1.5 mg	Working potential	+0.25 V	+0.70 V
Glutaraldehyde	10 µl	10 µl			

TABLE IV
Performance factors of the enzyme electrodes

Performance factors		
	B-MCPEE	PVF-MCPEE
Linear working range	1. Range: 1.9×10^{-8}–2.8×10^{-7} M	1. Range: 7.4×10^{-8}–2.8×10^{-7} M
	2. Range: 4.8×10^{-6}–2.8×10^{-5} M	2. Range: 5.0×10^{-6}–9.0×10^{-5} M
	3. Range: 3.5×10^{-5}–4.9×10^{-4} M	3. Range: 3.0×10^{-4}–7.0×10^{-3} M
	4. Range: 6.3×10^{-4}–2.7×10^{-3} M	
Limit of detection	1.9×10^{-8} M	7.4×10^{-8} M
Repeatability	4.7%	6.2%
Response time	150 s (t_{95})	120 s (t_{95})
Storage stability	12 days	14 days

TABLE V
Characteristics of various amperometric uric acid enzyme electrodes

No	Enzyme/Mediator/ Working potential	Immobilization matrix and Immobilization technique	Response time	Storage stability	Linearity/Detection limit/ Sensitivity	Buffer/pH/ Temperature	Ref.
1	UOX/Potassium ferricyanide/–200 mV	Dithiothreitol modified two layered carbon paste screen printed electrode/Adsorption	11 s	–	5.9×10^{-5}–1.2×10^{-3} M/ 7.7×10^{-6} M/ 173.46 µA mM^{-1} cm^{-2}	Borate/8.5/ 18–28 °C	4
2	UOX/–/+400 mV	Platinum-polyaniline electrode/ UOX doped into polyaniline film electrochemically	5 s	18% activity loss in 157 days	1×10^{-6}–1×10^{-3} M /–/–	B-R/7.0/ 39 °C	34
3	UOX/–/–700 mV	Polystyrene and polymaleimidostyrene modified gold disc electrode/ Adsorption	<1 min	13% loss in 21 days	5×10^{-6}–1×10^{-4} M /–/–	0.01 M Phosphate/8.5/ 30 °C	18
4	UOX/Microperoxidase-1/–100 mV	2-(2-mercaptoethylpyrazine), 4-4'-dithiodibutyricacid, Microperoxidase-11 and chitosan modified gold electrode/Adsorption	10 s (low conc.) 80–100 s (high conc.)	–	5×10^{-6}–1.5×10^{-4} M/ 2×10^{-6} M/ 3.4 ± 0.08 nAcm^{-2} µM^{-1}	0.05 M Phosphate/8.5/ room temperature	39
5	UOX/–/–	Uricase immobilized eggshell membrane /Cross-linking	<100 s	Min. 3 months	4×10^{-6}–6.4×10^{-4} M/ 2×10^{-6} M/–	0.2 M Phosphate/8.0/ 30 °C	38
6	UOX/Ferrocene/ +700 mV	Platinum-polypyrrole-ferrocenium electrode/Cross-linking	330 s	51% activity loss after 7 weeks	1×10^{-6}–5×10^{-5} M/ 5×10^{-7} M/–	0.05 M Borate/8.0/ 55 °C	23
7	UOX/–/+400 mV	Polyaniline film deposited indium-tin-oxide coated glass plate/ Cross-linking	60 s	5% loss after 17–18 weeks	1×10^{-5}–5×10^{-5} M/–/ 47.2 mAmM^{-1} (1.range) 1×10^{-4}–6×10^{-4} M/–/ 10.66 mAmM^{-1} (2.range)	0.05 M Tris HCl/6.5/ 35 °C	37

TABLE V
(*Continued*)

No	Enzyme/Mediator/ Working potential	Immobilization matrix and Immobilization technique	Response time	Storage stability	Linearity/Detection limit/ Sensitivity	Buffer/pH/ Temperature	Ref.
8	UOX-HRP/ferrocene-monocarboxylic acid/ +50 mV	Cylinditical pencil lead/ Cross-linking	–	10 days	5.9×10^{-7}–1.2×10^{-4} M/ 5.9×10^{-7} M/–	0.1 M Phosphate/ 7.5/ room temperature	17
9	UOX/–/+250 mV	Ir modified carbon electrode/ Cross-linking	41 s	–	1×10^{-4}–8×10^{-4} M/ 1×10^{-5} M/16.60 µAmM^{-1}	Phosphate/ 7.0/37 °C	21
10	UOX/Tetracyanoquinone-dimethane/+340 mV	Tetracyanoquinonedimethane modified carbon paste electrode/ Entrapment	70 s	13% loss in 4 months	1×10^{-6}–1×10^{-4} M/ 1.9×10^{-7} M/–	0.1 M Tris HCl/ 8.6/ room temperature	2
11	UOX-HRP/–/–	Teflon membrane covered dissolved oxygen probe/ Cross-linking	–	83% of initial activity remained in 1 month	1×10^{-7}–5×10^{-7} M/–/–	0.05 M Phosphate / 7.5/35 °C	15
12	UOX/1,4-benzoquinone/ +250 mV	Carbon paste/ Cross-linking	150 s	12 days	1.9×10^{-8}–2.7×10^{-3} M/ 1.9×10^{-8} M	0.20 M Tris HCl/ 8.0/ /30 °C	Presented electrode
13	UOX/PVF/+300 mV and +700 mV	Carbon paste/ Cross-linking	120 s	14 days	7.4×10^{-8}–7.0×10^{-3} M/ 7.4×10^{-8} M	0.05 M Phosphate / 8.0/40 °C	Presented electrode

interferences can be eliminated easily by the dilution of the sample and both electrodes showed very low detection limits (1.9×10^{-8} and 7.8×10^{-8} M) which allows uric acid determination in diluted samples. Table V shows the analytical characteristics of various amperometric uric acid enzyme electrodes reported in literature along with those obtained for the new modified enzyme electrodes proposed in this study.

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