Scientific paper

Amperometric Enzyme Electrodes for Xanthine Determination with Different Mediators

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Abstract

Two new amperometric carbon paste enzyme electrodes were developed for xanthine determination. 1,4-benzoquinone and poly(vinylferrocene) (PVF) were investigated as mediators. The parameters affecting the analytical performance of the enzyme electrode have been investigated in detail and optimized for modified enzyme electrodes. 1,4-benzoquinone modified enzyme electrode (BQ–CPEE) exhibited linear response from 1.9×10^{-7} M to 5.5×10^{-6} M and from 5.2×10^{-5} M to 8.2×10^{-4} M with a good detection limit of 1.0×10^{-7} M. The linear working range of the PVF modified enzyme electrode was between 1.9×10^{-7}–2.1×10^{-6} M, 1.9×10^{-6}–1.0×10^{-5} M and 1.1×10^{-4}–8.8×10^{-4} M with a detection limit of 1.0×10^{-7} M. Hypoxanthine response of the electrodes was also determined. Modified enzyme electrodes were used for xanthine determination in real samples and good recoveries were obtained.

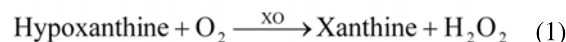
Keywords: Xanthine, Amperometry, Enzyme electrode, Xanthine oxidase, 1,4-benzoquinone, Poly(vinylferrocene)

1. Introduction

Xanthine (3,7-dihydro-1H-purine-2,6-dione) is a purine base found in most human body tissues and fluids and in other organisms. Xanthine is a product on the pathway of purine degradation. In clinical diagnostics the concentration of xanthine in blood and urine is used as an indicator for several pathologies such as xanthinuria, cerebral ischemia, renal failure and gout.[1–3] Xanthinuria is a rare genetic disorder where the lack of xanthine oxidase leads to high concentration of xanthine in body fluids and it can produce severe damages mainly related to renal failure. The concentration of xanthine is also used as an index for evaluating the fish freshness in food industry.[4] Thus the sensitive and selective determination of xanthine has considerable importance in clinical analysis and food quality control.

Various methods such as HPLC,[5] capillary electrophoresis-electrochemical analyses,[6] chemiluminescence,[7] and spectrophotometry[2] have been reported for quantification of xanthine and hypoxanthine. However, these methods are usually laborious, expensive, time-consuming and complex to perform. An alternative method for xanthine determination is the use of electrochemical enzyme electrodes, which allow direct, rapid and inexpensive measurement of xanthine in samples. Various types of enzyme electrodes have been reported for xanthine determination.[8–10]

Xanthine oxidase is a metal containing flavoprotein. It contains two molecules of flavin adenine dinucleotite, two atoms of molybdenum and eight atoms of nonheme iron[11]. Xanthine oxidase (XO) specifically catalyses the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid according to the following reactions.[3]



$$\text{Hypoxanthine} + O_2 \xrightarrow{XO} \text{Xanthine} + H_2O_2 \quad (1)$$

$$\text{Xanthine} + O_2 \xrightarrow{XO} \text{Uric acid} + H_2O_2 \quad (2)$$

In xanthine enzyme electrodes xanthine level can be determined electrochemically by measuring oxygen consumption or the concentration of H_2O_2 and/or uric acid produced by the enzymatic reaction. Xanthine determination based on H_2O_2 monitoring is more convenient due to the fact that biological fluids usually contain high level of O_2. However it is the high potential applied on the working electrode which makes enzyme electrodes responsive to uric acid and other interfering substances. The selectivity of xanthine enzyme electrodes can be improved by using redox mediators that permit a reaction

control at potentials lower than these required for H_2O_2 or uric acid oxidation.[9] It is known that quinones and ferrocene derivatives serve as electron acceptors in the reaction of flavoproteins.[12,13] The use of redox mediators in carbon paste electrodes is a promising approach.[14,15] The proposed system in this work is based on the amperometric monitoring of H_2O_2 which liberates during enzymatic reaction, at low applied potentials by using redox mediators. We constructed two different modified carbon paste enzyme electrodes by the incorporation of xanthine oxidase and redox mediator poly(vinylferrocene) or 1,4-benzoquinone within a carbon paste matrix and we investigated; the parameters that influence the electrode performance, the analytical characteristics, operational and storage stability, interference effects and the use of the enzyme electrodes for xanthine determination in real samples.

2. Experimental

2. 1. Equipment and Reagents

The electrochemical studies were carried out with IVIUM electrochemical analyzer (Ivium Technologies, Netherlands) and a three-electrode cell stand (Bioanalytical Systems, Inc., USA). The working electrode was a modified carbon paste electrode. The counter and the reference electrodes were a Pt wire (BAS MW 1034) and Ag/AgCl (BAS MF 2052) electrode respectively (Bioanalytical Systems, Inc., USA).

Xanthine oxidase (E.C.1.17.3.2. from *Microbial sp.* with a specific activity of 7 Units/mg solid), uric acid, xanthine, 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 and glucose were from Fluka (Buchs, Switzerland). Vinylferrocene and aspartic acid were from Aldrich (Steinheim, Germany). All other chemicals were obtained from Merck (Darmstadt, Germany). All solutions were prepared with bidistillated water. PVF was prepared by the chemical polymerization of vinylferrocene.[16] All the measurements were carried out at room temperature (23±2 °C).

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

1,4-benzoquinone modified carbon paste enzyme electrode (BQ-CPEE) and PVF modified carbon paste enzyme electrode (PVF-CPEE) were prepared by mixing the desired amounts of graphite powder (56.5%) 1,4-benzoquinone or PVF (8.7%) and paraffin oil (34.8%) with enzyme solution (50 μL xanthine oxidase (0.8 U), 1.5 mg BSA and 10 μL 1.25% glutaraldehyde). After mixing the paste for approximately 20 minutes to ensure the homogenity, the paste was packed firmly 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. Graphite powder (65.2%) and paraffin oil (34.8%) were mixed with the enzyme solution in a similiar way for unmodified enzyme electrode (UCPEE) construction. Enzyme electrodes were washed with distillated water and working buffer between measurements. Electrodes were stored in refrigerator at +4 °C when not in use.

2. 3. Amperometric Measurements

All amperometric measurements with BQ-CPEE were performed in phosphate buffer solution (0.05 M pH 7.5). We investigated the electrochemical oxidation of xanthine at UCPEE and BQ-CPEE. 5.0 mL phosphate buffer solution was added to the cell. After application of +0.25 V potential vs. Ag/AgCl, the background current was allowed to decay a constant value. Then an aliquot of 10^{-3} M xanthine stock solution was added to the cell and the current difference values versus xanthine concentrations were plotted in order to determine whether the electrode was sensitive to xanthine. The same experiment was performed for PVF-CPEE in 0.10 M pH 7.0 phosphate buffer solution at +0.60 V and +0.30 V. The cyclic voltammograms of BQ-CPE and PVF-CPE were recorded at (–0.5) V–(+0.9) V, in phosphate buffer solution at a scan rate of 50 mVs^{-1}.

3. Results and Discussion

3. 1. Xanthine Responses of Unmodified and Modified Carbon Paste Enzyme Electrodes

Cyclic voltammograms of the electrodes in the absence and presence of hydrogen peroxide are shown in Figure 1a and b. Figure 1a shows that a working potential of +0.25 V is convenient for BQ-CPEE. According to Figure 1b the optimum working potential of PVF-CPEE is +0.50 V. The amperometric response of PVF-CPEE was also determined at different working potentials between (0) – (+0.70) V by plotting calibration graphs to confirm this data. In the range from (+0.25) – (+0.60) V sensitivity was increased and the best sensivity was obtained at +0.60 V. Thus +0.60 V was selected as the optimum working potential for PVF-CPEE. However, we also obtained calibration curves with good linearity at +0.30 V with lower sensitivity for PVF-CPEE. This low potential is important to eliminate the possible interferences of other oxidizable species and to overcome the direct oxidation of uric acid.[25–27]

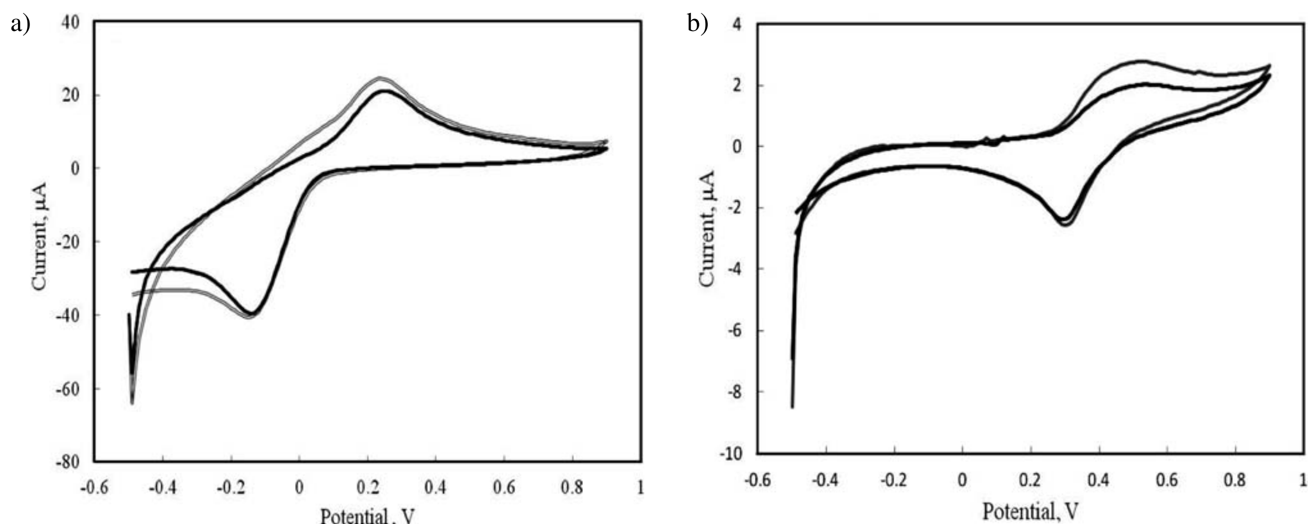


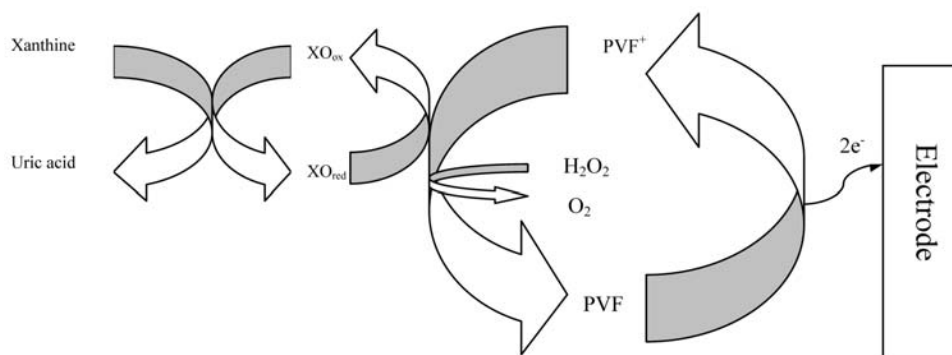
Figure 1. Cyclic voltammograms of the (a)BQ-CPE in the absence (black line) and in the presence of 0.1 mM H_2O_2 (grey line) (b)PVF-CPE in the absence (black line) and in the presence of 0.1 mM H_2O_2 (grey line) in phosphate buffer solution at 50 mVs^{-1}.

We investigated the xanthine response of the modified and unmodified electrodes. Xanthine sensitivity of the BQ-CPEE (6.91 µA mM^{-1}) was found to be much higher than that of UCPEE (8.6×10^{-1} µA mM^{-1}) at +0.25 V. Xanthine sensitivity of PVF-CPEE (4.61 µA mM^{-1}) was also higher than that of UCPEE (9.4×10^{-1} µA mM^{-1}) at +0.30 V. The sensitivity of PVF-CPEE was higher than UCPEE also at +0.60 V. It can be concluded that both 1,4-benzoquinone and poly(vinylferrocene) act as electron transfer mediators and can help in enhancing the sensitivity of enzyme electrodes. Electrons generated from the biochemical reaction would transfer to the modified enzyme electrodes through the PVF^+ /PVF or Q/H_2Q couples. 1,4-Benzoquinone reduces to hydroquinone (H_2Q) and PVF^+ to PVF. Hydroquinone at +0.25 V and PVF at +0.60 V are also electrooxidized on carbon paste electrode surface and the oxidized forms, 1,4-benzoquinone and PVF^+ are re-formed. Our results are found to be in good agreement with the data reported.[14,17,18]

The reaction scheme for the PVF-CPEE can be illustrated as follows:

3. 2. Optimum Working Conditions and Electrode Composition of BQ-CPEE and PVF-CPEE

The amperometric response of an enzyme electrode greatly depends on the amount of the enzyme loaded. The best composition of the carbon paste was determined by comparing the sensitivity and working ranges of the electrodes for different enzyme and mediator amounts. The response of the BQ-CPEE was measured at four different enzyme amounts as 0.4 Units; 0.8 Units; 1.2 Units and 1.6 Units by keeping the other parameters constant. An increase in the sensitivity was observed as the amount of the enzyme increased from 0.4 Units to 0.8 Units and decreases afterwards. Maximum sensitivity was observed at the loading of 0.8 Units. This enzyme amount was also used for PVF-CPEE. Moreover, over 1.2 Unit of enzyme, the response current tends to be saturated and no significant increase in the sensitivity was observed. The optimum enzyme loading was specified as 0.8 Unit because further increase of enzyme loading would be a waste of this expensive reagent.



A study was carried out to evaluate the effect of 1,4-benzoquinone amount in the carbon paste matrix on the electrode response. Mediator amount was varied as 4.3%, 6.5%, 8.7%, 10.9% and 13.0% while the graphite and paraffin oil amount kept constant. The highest sensitivity and working range was obtained with the carbon paste electrode with 8.7% 1,4-benzoquinone. Similar to the enzyme amount parameter, the response current tends to be saturated and no significant increase in the sensitivity was observed with 10.9% and 13.0% mediator composition. This mediator amount was also used for PVF-CPEE.

Tris, phosphate and borate buffers were investigated for the performance of BQ-CPEE and PVF-CPEE. The sensitivities of BQ-CPEE and PVF-CPEE were higher in phosphate buffer than the other buffers. Therefore, phosphate buffer was selected as the optimum buffer type and all the following measurements were performed at this buffer. The amperometric response of BQ-CPEE was determined at different phosphate concentrations of 0.05 M; 0.10 M; 0.15 M and 0.20 M and the best response was obtained at 0.05 M. Above or below this concentration, the response was found to show a significant decrease. The amperometric response of PVF-CPEE was also determined in the same phosphate concentrations and best response was obtained at 0.10 M.

The optimum pH range is critical for many enzymatic reactions, thus the effect of pH on the response of BQ-CPEE and PVF-CPEE was investigated at various pH values. Highest sensitivity was obtained at pH 7.5 and pH 7.0 for BQ-CPEE and PVF-CPEE, respectively (Fig.2). The reported optimum pH of xanthine oxidase is in the range of 7.0–8.5.[19,20] We can say that immobilization procedure has no significant influence on the properties of xanthine oxidase. These values are in good agreement with the data reported.[4,10,21–23] pH values different than 7.0 and 7.5 were also reported for xanthine enzyme electrodes; pH 6.8,[9] pH 7.4, [13] pH 8.6.[24] This was attributed to the fact that the mediator, enzyme supply, immobilization method and electrode preparation procedures were different. The sharp decrease in the response of the enzyme electrodes at higher pH values might be due to the poor enzyme activity.

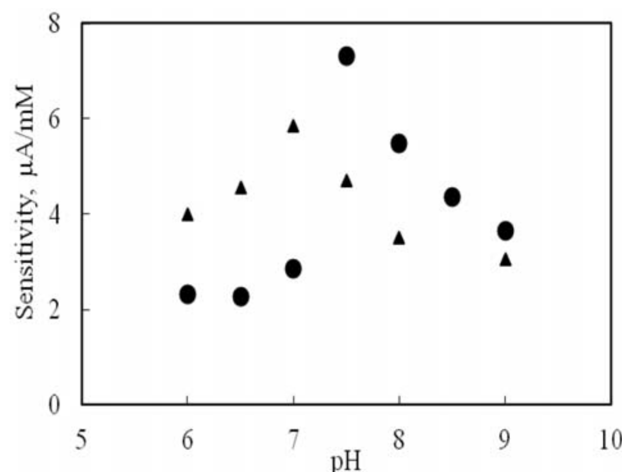


Figure 2. The effect of solution pH on the response of the electrodes ●: BQ-CPEE 0.05 M phosphate buffer solution, +0.25 V; ▲: PVF-CPEE 0.10 M phosphate buffer solution +0.30 V, room temperature.

3. 3. Performance Parameters of BQ-CPEE and PVF-CPEE

The response time of the enzyme electrode depends on the xanthine concentration thus the amperometric response time of BQ-CPEE and PVF-CPEE to xanthine was determined at two different concentrations. The current values for 1.0×10^{-5} M and 5.0×10^{-5} M xanthine versus time were plotted (Fig.3). The response time was shorter at lower concentrations than that at higher concentrations. The time required to reach 95% of the steady-state current was about 100 s (t_{95}) for BQ-CPEE and 50 s (t_{95}) for PVF-CPEE. This response time is quite fast and highly suitable for biosensor response. There are longer; 3–4 min[13] and shorter response times 5 s,[28] 6 s,[21] 14 s,[10] 50 s,[4] reported for xanthine enzyme electrodes.

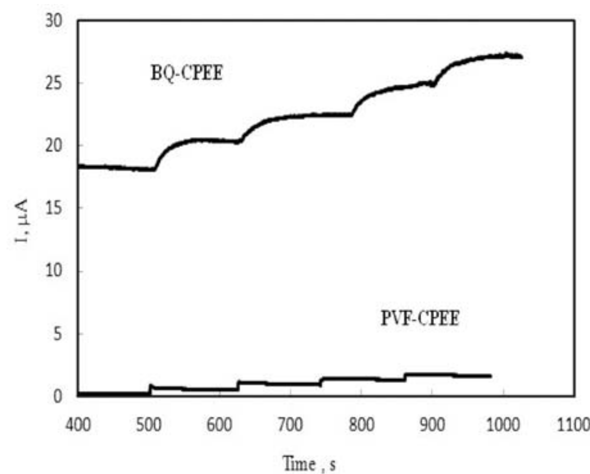


Figure 3. Current-time curves of (a) BQ-CPEE and (b) PVF-CPEE upon successive additions of 1.0×10^{-5} M and 5.0×10^{-5} M xanthine

The repeatability of BQ-CPEE and PVF-CPEE were investigated. Five calibration curves were plotted by the use of the same electrode sequentially. The relative standard deviation of the sensitivities (the slopes of the curves) was found to be 3.5% for BQ-CPEE and 7.2% for PVF-CPEE. This result indicates that the repeatability of the enzyme electrodes is highly satisfactory and electrodes can be used for many analyses.

Figure 4 shows the amperometric response of the BQ-CPEE recorded as a function of xanthine concentration under optimum conditions. The limit of detection of

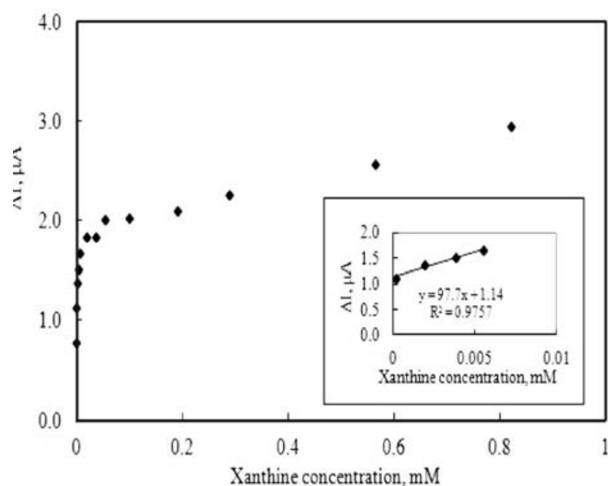


Figure 4. The effect of xanthine concentration on the response of BQ-CPEE (inset) The response of the electrode to xanthine at the lower concentration region (0.05 M, pH 7.5 phosphate buffer, +0.25 V, room temperature)

the enzyme electrode is 1.0×10^{-7} M. There are two linear parts ranging from 1.9×10^{-7} M to 5.5×10^{-6} M and from 5.2×10^{-5} M to 8.2×10^{-4} M. The regression equation of the first linear part of the curve is $\Delta I = 97.7c_{xanthine} + 1.14$ ($R^2 = 0.9757$) and the second is $\Delta I = 1.24c_{xanthine} + 1.9$ ($R^2 = 0.992$).

We also investigated the response of the BQ-CPEE to hypoxanthine (Fig. 5) and the electrode showed linear response to hypoxanthine between 2.9×10^{-7}–7.2×10^{-6} M and 1.7×10^{-4}–1.5×10^{-3} M.

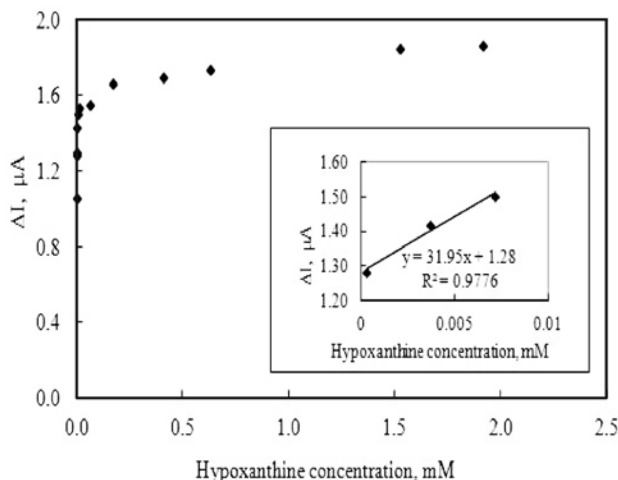


Figure 5. Effect of hypoxanthine concentration on the response of BQ-CPEE (inset) The response of the electrode to hypoxanthine at the lower concentration region (0.05 M, pH 7.5 phosphate buffer, +0.25 V, room temperature)

A linear relationship was observed between the amperometric response and xanthine concentration from 1.9×10^{-6} M to 1.0×10^{-5} M and from 1.1×10^{-4} M to 8.8×10^{-4} M for PVF-CPEE at +0.30 V (Fig. 6). We also investigated the response of the PVF-CPEE at lower concentrations and the enzyme electrode showed linear response between 1.9×10^{-7}–2.1×10^{-6} M with a detection limit of 1.0×10^{-7} M. The response at lower concentrations is important when working with diluted samples to eliminate the interference effects. PVF-CPEE also showed linear response to xanthine between 9.7×10^{-7}–9.1×10^{-6} M and 1.1×10^{-4}–1.6×10^{-3} M at +0.60 V. Hypoxanthine response of PVF-CPEE was linear between 1.0×10^{-5} M–3.0×10^{-4} M at +0.30 V. Our results show that, there is a remarkable improvement in detection limit and linear working range of the modified enzyme electrodes when compared to other xanthine biosensors recently reported. [9,10,12]

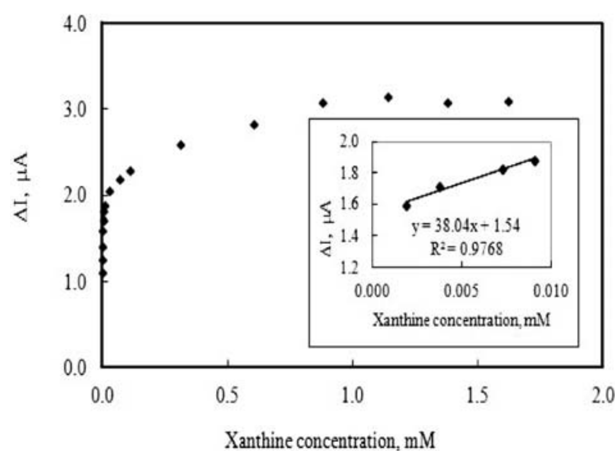


Figure 6. Effect of xanthine concentration on the response of PVF-CPEE (inset) The response of the electrode to xanthine at the lower concentration region (0.10 M pH 7.0 phosphate buffer, +0.30 V, room temperature)

The storage stability of an enzyme electrode is an important characteristics concerning biosensor development. We checked the long-term stability of BQ-CPEE and PVF-CPEE prepared under optimum conditions. The electrodes were stored at +4 °C under dry atmosphere when not in use. Calibration curves were plotted at different days during the storage period and the BQ-CPEE lost 34% of its initial sensitivity after 18 days. PVF-CPEE showed 88% of initial activity after 6 days and 33% after 21 days. The decrease in the sensitivity of the enzyme electrodes can be attributed to the fact that enzymes lose activity by time. PVF-CPEE loses 67% of its initial activity after 21 days but BQ-CPEE loses 34% of its initial activity after 18 days so it can be concluded that storage stability of BQ-CPEE is better than the PVF modified one.

3. 4. Effect of Interferences

There are a variety of interferents coexisting in biological samples hence purposed enzyme electrodes should

have significant specificity against these coexisting substances.[29] Effect of ascorbic acid, uric acid, glucose, urea, creatinine, methionine and aspartic acid on the response current was investigated at a constant xanthine concentration of 2.0×10^{-5} M. The concentrations of the interferences were selected similar and below their physiological concentration. Table 1 shows the specifity of the BQ-CPEE and PVF-CPEE for various concentrations of biological substances normally present in human serum and urine. Ascorbic acid is considered to be the major interferant in biological samples and in our study it causes a significant interference of 18.20% at its highest concentration found in urine. However, when concentration of ascorbic acid is decreased the effect of interference reduces. This case is the same 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.[30,31] Both of the electrodes ensure improved selectivity since no significant response was observed in the presence of interfering species at low concentrations.

Table 1: Effect of interferences on the response of modified electrodes.

Interfering species	Concentration of the interference (M)	Interference % (BQ-CPEE)	Interference % (PVF-CPEE)
Ascorbic acid	3×10^{-4}	18.20	18.38
	1×10^{-4}	–1.64	7.97
	1×10^{-5}	–	–1.54
Glucose	4×10^{-3}	–10.60	–11.26
	1×10^{-4}	–1.55	3.91
Creatinine	8×10^{-4}	–4.33	6.48
	1×10^{-4}	–1.70	–2.45
Urea	8×10^{-4}	0.94	–6.92
	5×10^{-2}	–18.52	10.48
Methionine	5×10^{-8}	0.27	0.31
	4×10^{-5}	21.58	–22.20
Aspartic acid	5×10^{-3}	–28.75	6.65
	1×10^{-5}	–3.14	4.14
Uric acid	3×10^{-4}	3.59	4.22
	1×10^{-4}	2.51	3.45

3. 5. Determination of Xanthine in Urine

The practical application of the developed enzyme electrodes was established by the determination of xanthine in human urine. The urine samples were diluted without any other pretreatment process. The xanthine concentration of the urine samples were determined by standard addition method. The mean recoveries of the spiked samples were 101.5% and 97.9% for BQ-CPEE and PVF-CPEE respectively (Table 2). From these recovery values it is concluded that proposed enzyme electrodes are highly accurate.

Table 2: Determination of xanthine in human urine samples using modified electrodes (n = 5)

Sample	Electrode	Xanthine added (μM)	Xanthine found (μM)	Mean Recovery%
1	BQ-CPEE	0	8.54	–
		43	50.9±1.1	98.8
2	BQ-CPEE	0	5.13	–
		60	67.9±1.4	104.2
1	PVF-CPEE	0	8.38	–
		43	50.5±1.3	98.3
2	PVF-CPEE	0	5.97	–
		60	64.3±1.3	97.5

Table 3: 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	8.7%	8.7%	Buffer	Phosphate	Phosphate
Graphite amount	56.5%	56.5%	pH	7.5	7.0
Parafine oil	34.8%	34.8%	Buffer Concentration	0.05 M	0.10 M
Enzyme amount	0.8 U	0.8 U	Working Potential	+0.25 V	+0.30 V and +0.60 V
BSA	1.5 mg	1.5 mg			
Glutaraldehyte	10 μL	10 μL			

Performance Factors	BQ-CPEE	PVF-CPEE
Linear Working Range	1. Range: $1.9 \times 10^{-7} – 5.5 \times 10^{-6}$ M (xanthine)	1. Range: $1.9 \times 10^{-7} – 2.1 \times 10^{-6}$ M (xanthine)
	2. Range: $5.2 \times 10^{-5} – 8.2 \times 10^{-4}$ M (xanthine)	2. Range: $1.9 \times 10^{-6} – 1.0 \times 10^{-5}$ M (xanthine)
	1.Range: $2.9 \times 10^{-7} – 7.2 \times 10^{-6}$ M (hypoxanthine)	3. Range: $1.1 \times 10^{-4} – 8.8 \times 10^{-4}$ M (xanthine)
	2.Range: $1.7 \times 10^{-4} – 1.5 \times 10^{-3}$ M (hypoxanthine)	1. Range: $1.0 \times 10^{-5} – 3.0 \times 10^{-4}$ M (hypoxanthine)
Limit of Detection	1.0×10^{-7} M (xanthine)	1.0×10^{-7} M (xanthine)
Repeatability	3.5%	7.2%
Response Time	100 s (t_{95})	50 s (t_{95})
Storage Stability	14 days	7 days

Table 4: Characteristics of various amperometric xanthine and hypoxanthine enzyme electrodes.

No	Enzyme/Mediator/Working potential	Immobilization matrix/ Immobilization technique	Response time/ Repeatability	Storage stability	Linearity/ Detection limit /Sensitivity	Buffer /pH/ temperature	Ref.
1	XO/–/–0.4 V vs. Ag/AgCl	Glassy carbon electrode modified with multiwalled carbon nanotube	6 s/ RSD 3.4% (*n* = 7)	5% loss after 90 days.	2×10^{-7}–1×10^{-5} M/ 1×10^{-7} M/– (Xanthine)	0.05 M p hosphate/7.0/ room temperature	[1]
2	XO/–/+0.55 V vs.SCE (H_2O_2 oxidation) XO-HRP/–/–0.50 V vs.SCE (H_2O_2 reduction)	Nano $CaCO_3$ particles and XO modified electrode/ cross-linking Nano $CaCO_3$ particles, XO and HRP modified electrode /cross-linking	<5 s/ RSD 4.9% (*n* = 61) (XO-Nano $CaCO_3$)	15% loss after 28 days (XO-Nano $CaCO_3$)	2.0×10^{-6}–2.5×10^{-4} M/2.0×10^{-6} M171.3 mAM^{-1} (XO-Nano$CaCO_3$ electrode) 4.0×10^{-7}–5.0×10^{-5} M/1.0×10^{-7} M/– (XO-HRP Nano$CaCO_3$ electrode)	0.05 M phosphate /7.5/ room temperature	[28]
3	XO/Colloidal gold/+0.70 V vs.Ag/AgCl	Glassy carbon electrode modified with colloidal gold /XO mixed with carbon paste	50 s/ RSD 6.31% (*n* = 5) (xanthine) 3.57% (*n* = 5) (hypoxanthine)	18% loss after 1 week	5×10^{-7}–1×10^{-5} M /– /–(xanthine) 5×10^{-6}–$1{,}5 \times 10^{-4}$ M /– /–(hypoxanthine)	0.05 M Phosphate/7.5/ room temperature	[4]
4	XO/–/ +0.70 V vs. Ag/AgCl	XO was immobilized on gold electrode modified with β-cyclodextrin branched carboxymethycellulose and covered with 1- adamantanyl layer	14 s/	7% loss after 3 weeks	3×10^{-4} M-1×10^{-2} M/2×10^{-4} M/ 8.2 mA/M cm^{-2} (xanthine)	0.1 M phosphate /7.0/room temperature	[10]
5	XO/–/ +0.60 V and 0 V vs. Ag/AgCl	Carbon paste electrode modified with gold nanoparticles/Cross-linking	–/RSD 3.9% (*n* = 10)	15 days	5.0×10^{-6}–2.5×10^{-5} M/2.2×10^{-7} M/– (hypoxanthine)	0.05 M phosphate/7.4/ room temperature	[32]
6	XO/–/ –0.5 V vs. Ag/AgCl	Graphite electrode modified with platinum and ve palladium microparticles /Adsorption	<2 min	35% loss after 30 hours	Linear up to 4×10^{-5} M/ 4.5×10^{-6} M/0.21 μAμM^{-1} (xanthine)	Phosphate/8.4/ 23 °C	[33]
7	XO/sodyum montmorillonit-metil viyolojen/	Carbon paste electrode modified with sodium montmorillonite-methyl viologen/ Electropolymerization	–/–	60% loss after 5 weeks	1×10^{-6}–4×10^{-4} M /8×10^{-7} M/ (hypoxanthine)	Phosphate/7.0/ 30–38 °C	[34]
8	XO/poly(mercapto-p-benzoquinone)/+0.30 V vs Ag/AgCl	Glassy carbon electrode modified with gold and poly (mercapto-p-benzoquinone/ Electropolymerization	<1 min.	30% loss after 8 days	1×10^{-6}–8×10^{-5} M /–/– (xanthine) 1×10^{-6}–5×10^{-5} M /–/– (hypoxanthine)	Phosphate/7.3/ 30 °C	[12]

4. Conclusion

In this study determination of xanthine in urine samples was carried out using BQ-CPEE and PVF-CPEE. The properties and optimum working conditions of the carbon paste enzyme electrodes are summarized in Table 3. The sensitivity and selectivity of the presented electrodes demonstrates their practical applicability for a simple, rapid and low cost determination of xanthine. It can be concluded that both 1,4-benzoquinone and poly(vinylferrocene) mediators have served to prepare modified amperometric enzyme electrodes for reliable determination of xanthine. 1,4-Benzoquinone allowed the determination of xanthine occur at a low potential (+0.25 V) hence reducing the risk of interference. By the use of PVF xanthine determination was achieved at +0.30 V. In this study it was shown that effect of interferences can be eliminated easily by the dilution of the sample and working at low potentials. The advantages of the proposed modified enzyme electrodes such as low cost, simple fabrication procedure, fast response, low detection limit, good accuracy and good repeatability make them suitable for routine analysis of xanthine. Table 4 shows the analytical caracterictics of various amperometric xanthine enzyme electrodes reported. The developed enzyme electrodes have the advantage of wider linear working range, lower detection limits for xanthine and hypoxantine and low working potentials when compared with the other enzyme electrodes.

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Povzetek

Razvili smo dve novi amperometrični ogljikovi encimski elektrodi za določanje ksantina. Kot mediatorja smo preizkusili 1,4-benzokinon ter poli(vinilferocen) – PVF. Podrobno smo raziskali parametre, ki vplivajo na analizno učinkovitost encimskih elektrod, ter jih optimizirali za modificirane encimske elektrode. Encimska elektroda modificirana z 1,4-benzokinonom (BQ-CPEE) je imela linearen odziv od $1,9 \times 10^{-7}$ M do $5,5 \times 10^{-6}$ M in od $5,2 \times 10^{-5}$ M do $8,2 \times 10^{-4}$ M z dobro mejo zaznave pri $1,0 \times 10^{-7}$ M. Linearno delovno območje encimske elektrode modificirane s PVF je bilo med $1,9 \times 10^{-7}$ M–$2,1 \times 10^{-6}$ M, $1,9 \times 10^{-6}$ M–$1,0 \times 10^{-5}$ M in $1,1 \times 10^{-4}$ M–$8,8 \times 10^{-4}$ M z mejo zaznave $1,0 \times 10^{-7}$ M. Določili smo tudi odziv elektrod na hipoksantin. Modificirane encimske elektrode smo uporabili za določitev ksantina v realnih vzorcih z dobrimi izkoristki oz. pravilnostjo.