Full Paper

Electrochemical Determination of Oxalate at Pyrolytic Graphite Electrodes

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Abstract
The electrocatalytic oxidation of oxalate at several carbon based electrodes including basal plane (BPPG) and edge plane (EPPG) pyrolytic graphite and glassy carbon (GC) electrode, was studied. The electrodes were examined for the sensing of oxalate ions in aqueous solutions and all three electrodes showed a response to oxalate additions. The peak of oxalate oxidation at BPPG electrode appeared at lower potential, +1.13 V vs. SCE, than at EPPG (+1.20 V vs. SCE) and GC electrode (+1.44 V vs. SCE). Oxalate oxidation at BPPG electrode was studied in more details for response characteristics (potential and current), effects of pH, temporal characteristics of response potential and current. The results indicated that oxalate oxidation proceeds as two-electron process at the BPPG electrode with a transfer coefficient β and a diffusion coefficient D evaluated to be 0.45 and 1.03 (± 0.04) $\times 10^{-5}$ cm^2 s^{-1} respectively. The BPPG electrode was found to be suitable for oxalate determination in aqueous media showing linear response to oxalate concentration with a sensitivity of 0.039 AM^{-1} and a limit of detection of 0.7 μM.

Keywords: Basal plane pyrolytic graphite electrode, Edge plane pyrolytic graphite electrode, Electrooxidation, Oxalate

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1. Introduction

The sensing of oxalates is an important issue in the fields of food chemistry and clinical analysis. The determination of oxalates in urine is of clinical importance in the diagnosis of urinary tract stones and hyperoxaluric acid syndromes. Oxalates can be ingested as dietary constituent or produced as the final product of amino and ascorbic acid metabolism. Oxalate ions are potentially toxic to humans because they form insoluble complexes with divalent cations and produce renal stones [1]. Calcium oxalate is the principal constituent of urinary tract stones in 60–80% of cases and the concentration of oxalic acid in urine is an important risk factor for calcium-containing stones. In addition to being excreted in excess in the hyperoxaluric syndromes, the mean urinary oxalate excretion is higher in patients with recurrent idiopathic stones than in normal subjects. Since even a small oxalate excess in an organism could lead to serious health problems determination of oxalate is a very important task that requires the search for simple, accurate, reliable and low cost methods for its determination.

Existing methods for oxalate determination include liquid and gas chromatography [2–6], capillary electrophoresis [7], spectrometry (UV-vis absorption, fluorimetry and atomic absorption spectroscopy) [8, 9] and flow-injection analysis [10, 11]. These methods are, however, expensive, time consuming, require pre-treatment and occasionally have a poor selectivity.

In order to overcome these disadvantages, enzymatic systems and biosensors have been developed [12]. Enzymatic systems based either on the pure oxalate oxidase enzyme [13] or on the naturally immobilized enzyme [14], employing conductimetric [15], spectrophotometric [16, 17], biothermochip [18], potentiometric [19] and amperometric detection [20–23] have been described for the determination of oxalate in different matrices, including biological samples. Amperometric biosensors, however, suffer from significant interference problems. Amperometric oxalate biosensors require monitoring of hydrogen peroxide oxidation current at very high potential values; this fact leads to the problem that the interference of the oxidation of other substances that could be present in the sample, must be eliminated. To overcome this problem and reduce the operating potential some use of two enzymes [24] and metallic hexacyanide complexes as the transductor of oxygen peroxide signal [21] has been reported.

Amperometric sensors are commonly very attractive and suitable for oxalate determination due to simple use, selective and fast determination of the analyte and the possibility of analyzing complex samples without pretreatment. The electrocatalytic oxidation of oxalate has been reported at metal electrodes such as platinum [25, 26] as well as metal modified carbon electrodes such as palladium based modified glassy carbon electrode [27], carbon paste electrode with deposited palladium nanoparticles [28] and highly boron doped diamond electrodes [29].

To the best of our knowledge, the electrochemical oxidation of oxalates at basal plane or edge plane pyrolytic graphite electrode has not yet been reported. The objective of the present study was to investigate the electrooxidation of oxalates at carbon based electrodes, basal plane and edge plane pyrolytic graphite and GC electrodes and to establish a method for quantifying oxalates by employing these electrodes. The BPPG electrode was found to allow very stable and sensitive oxalate determination, in a wide concentration range. Moreover, the characteristics of the cyclic voltammograms of oxalate with varying scan rate, pH using BPPG electrode are examined.

2. Experimental

2.1. Reagents and Chemicals

All chemicals used in this study were of analytical grade and used as received, without further purification. These were: sodium oxalate (AnalaR) and potassium sulfate (Aldrich). The buffers used at different pH values were: acetate buffers for pH 4.0 and 5.0, phosphate buffers for pH 2.0, 3.0, 6.0, 7.0 and 8.0 and boric buffer for pH 9.0 and 10.0. All solutions were prepared with deionized water of resistivity not less than 18.2 MΩ cm (Vivendi Water systems, UK). High-purity nitrogen gas (BOC, Guildford, Surrey, UK) was used to outgas the buffer solutions prior to the measurements.

2.2. Instrumentation

All electrochemical examinations were carried out in a single-compartment three-electrode cell with a solution volume of 10 cm^3. The working electrodes were edge plane pyrolytic graphite electrode (EPPG, 5 mm diameter, Le Carbone Ltd, Sussex, UK), a glassy-carbon electrode (GC, 3 mm diameter, BAS, Indiana, USA) or basal plane pyrolytic graphite electrode (BPPG, 5 mm diameter, Le Carbone Ltd, Sussex, UK). In all experiments, the counter electrode was a platinum electrode (3 mm diameter), with a saturated calomel electrode (SCE, Radiometer, Copenhagen, Denmark) completing the circuit. The measurements were performed at the constant temperature of 22 °C (±2 °C). Voltammetric studies were carried out and cyclic voltammograms recorded on a μ-Autolab (ECO-Chemie, Utrecht, The Netherlands) potentiostat.

Oxalate concentration was determined using linear sweep voltammetry (LSV). The electrochemical determination of analytes concentrations was performed via the standard addition protocol with a limit of detection evaluated using 3 sigma method.

3. Results and Discussion

Prior to the measurements, all electrodes were pretreated since the electrode history can determine to a great extent electrochemical performance [30]. Different pre-treatment procedures were applied depending on the electrode used. GC electrodes were polished with diamond lapping compounds (1 micron, Kelmet, UK) and then thoroughly rinsed with water. The electrode was then sonicated in nitric acid solution for 10 min. Before measurements, EPPG electrodes were polished to a mirror-like finish with 1.0 and 0.3 μm alumina slurry (Buehler). The electrode was then carefully rinsed with water to remove any alumina residue. A BPPG electrode surface was prepared for measurements with cellotape (TM) [31]. This involves first polishing the electrode surface on carborundum paper. Next cellotape was pressed on the clean electrode surface and removed along with several surface layers of graphite [31]. This process was repeated many times and electrode finally cleaned in acetone to remove any adhesive residue.

Electrooxidation of oxalate at different carbon electrodes: BPPG, EPPG and GC electrode, was studied using cyclic voltammetry (CV) in 1 mM oxalate solution with 0.1 M K_2SO_4 as supporting electrolyte at scan rate of 50 mV s^{-1} in the potential range 0 to 1.6 V vs. SCE. First, cyclic voltammograms of all three electrodes were run in 0.1 M K_2SO_4 with no oxalate added but no peak was observed in the potential range examined. A chemically irreversible peak corresponding to the oxidation of oxalate could be observed at CVs of all three electrodes upon the addition of oxalates (Fig. 1). However, the peak of oxalate oxidation observed at GC electrode appeared at high potential of +1.44 V vs. SCE. Oxalate oxidation peaks at BPPG and EPPG electrodes appeared at lower potentials, +1.13 and 1.20 V vs. SCE respectively, and with peak currents higher than one obtained at GC electrode. Carbon electrode reactivity in general is, depending on the analyte, influenced by surface cleanliness [32], surface microstructure [33], hydrophobicity/hydrophilicity [13], electronic structure, i.e., density states [34] and surface carbon oxides [35].

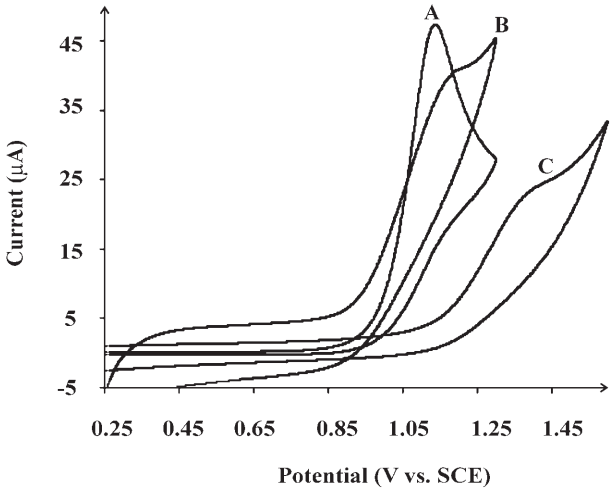

Fig. 1. Cyclic voltammograms of BPPG (A), EPPG (B), and GC (C) electrode in 1 mM oxalate solution with 0.1 M K_2SO_4 as supporting electrolyte at scan rate of 50 mV s^{-1}.

Next we turned to examining the electrooxidation of oxalate at BPPG electrode in more detail.

The effect of the scan rate on the electrooxidation of oxalate at BPPG electrode was subsequently investigated. Cyclic voltammograms of BPPG electrode were run in 1 mM oxalate solution in 0.1 M K_2SO_4 in the scan rate range of 5 to 500 mV s^{-1}. The current responses of oxalate oxidation at BPPG electrode was found to be proportional to square root of scan rate increasing in a straight line in the range of 5 – 400 mV s^{-1}. This indicated that the oxidation is under diffusion control in the interfacial area of the electrode, with no observable adsorption or specific surface processes occurring.

The effect of pH on the electrooxidation currents and potentials was investigated by cyclic voltammetry in 0.5 mM oxalate solutions in pH range from 0 to 8.0. Oxalates were oxidized at BPPG electrode both in acidic and alkaline solutions as evidenced by appearance of a clear oxidation peak at all pH values in the range of 1.0 to 8.0 (Fig. 2). The peak current is somewhat sensitive to pH variations. Peak potential decreased with increasing pH in the range from 1 to 4 unit, from +1.38 V in pH 1.0 solution to +1.113 V vs. SCE in pH 4 solution. The decrease of peak potential with pH in the range 1 to 4 reflects the known p*K*a values of oxalic acid of 1.25 and 4.20 (25 °C) [27]. At pH values higher than 4, the oxidation potentials were constant, indicating that the process was not pH-dependent.

$$H_2C_2O_4 \rightarrow H^+ + HC_2O_4^- \quad (1)$$

$$HC_2O_4^- \rightarrow H^+ + C_2O_4^{2-} \quad (2)$$

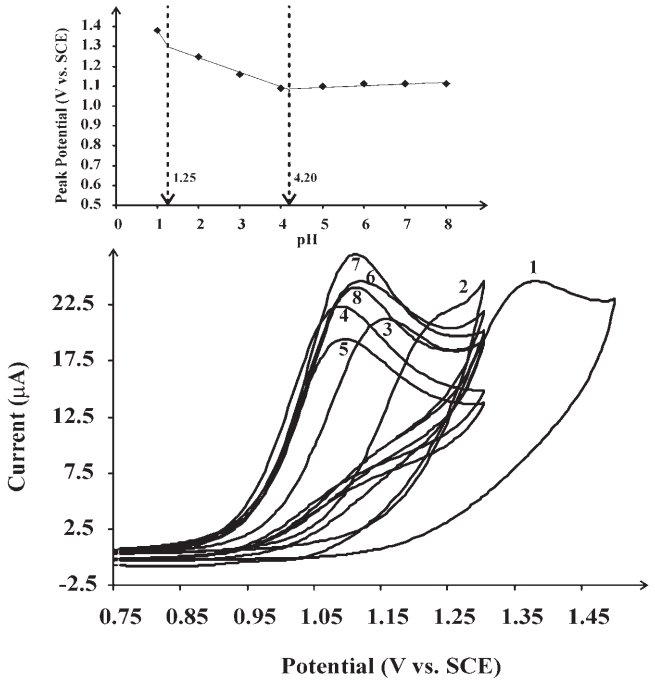

Fig. 2. Cyclic voltammograms of BPPG electrode in 0.5 mM oxalate solutions of different pH values at scan rate of 50 mV s^{-1} with peak potential versus pH plot (inset). The pK_a values of 1.25 and 4.20 (25 °C) are marked.

$$C_2O_4^{2-} - 2e \rightarrow 2\ CO_2 \quad (3)$$

It is interesting to note that no change in electrode activity was observed and the peak current and peak potential were the same for the first and the subsequent sweeps with the same electrode after brief stirring. This indicated that there was no BPPG electrode response degradation as it has been reported with noble metal electrodes [36 – 39]. With continuous cycling for 50 times in 1 mM oxalate solution at pH 7, only a 2% decrease in the peak current could be observed. This observation further supported the assumption that the oxidation of oxalate at the BPPG electrode is proceeding via a diffusionally controlled mechanism and not by a mechanism that would include an adsorption process. Indeed, mechanisms including adsorption step are likely to lead, in some extent, to the passivation or a modification of the active surface of the electrode after a great number of experiments.

Oxidation of oxalate at platinum and glassy carbon electrodes has previously been studied in dipolar aprotic organic solvents [40] and it was shown that it takes place following stepwise dissociative electron transfer mechanism. The electrochemical oxidation of oxalates is believed to proceed as a two-electron process producing carbon dioxide. This is in agreement with literature reports on electrooxidation of oxalates at platinum electrode in acetonitrile solution [41]. We assume that the electrooxidation of oxalate at BPPG electrode in aqueous solutions is following a similar mechanism:

$$C_2O_4^{2-} - e \rightarrow 2\ C_2O_4^{\cdot -} \quad (4)$$

$$C_2O_4^{\cdot -} \rightarrow CO_2 + CO_2^{\cdot -} \quad (5)$$

$$CO_2^{\cdot -} - e \rightarrow CO_2 \quad (6)$$

giving the overall Reaction 7:

$$C_2O_4^{2-} - 2e \rightarrow 2\ CO_2 \quad (7)$$

The transfer coefficient β is defined as:

$$\beta = (2.3\ RT/F)\ \partial \lg\ i_{ox}/\partial E \quad (8)$$

where R is universal gas constant, T temperature, F Faraday constant and i_{ox} oxidative current. The transfer coefficient was calculated using both the slope of Tafel logarithm of oxidative current i_{ox} versus potential E plot and from the half-peak width values. In both cases, the value of 0.45 for transfer coefficient β was evaluated indicating a stepwise electron transfer mechanism.

Diffusion coefficient D was evaluated using Randles – Ševčík equation for irreversible electron transfer:

$$i_p = (2.99 \times 10^5)\ n\ (\beta \cdot n_a)^{1/2}\ ACD^{1/2}\ v^{1/2} \quad (9)$$

where n is the number of electrons, n_a the number of electrons involved in the charge-transfer step, A the

electrode area (in cm^2), C the concentration (in mol cm^{-3}) and v the scan rate (in V s^{-1}). The diffusion coefficient was found to be 1.03 ($\pm$0.04) $\times 10^{-5}$ cm^2 s^{-1} using the calculated value of 0.45 for transfer coefficient β and assuming a two-electron process with one electron being involved in the charge-transfer step. This value is in agreement with value of diffusion coefficient estimated using the Wilke – Chang equation. This suggests that the two-electron process is taking place.

In real samples, interference of some organic acids as well as some phenols might occur. Ascorbic acid is one of the main metabolites that can interfere with oxalates determinations, but a good differentiation is expected at BPPG electrode. The ascorbic acid oxidation peak at BPPG electrode in 0.5 mM ascorbic acid solution in pH 7 phosphate buffer at scan rate of 50 mV s^{-1} appears at ca. +0.245 V vs. SCE, a value ca. 0.87 V more negative from the oxalate oxidation peak potential value. Cyclic voltammograms of BPPG were also run in 1 and 2.5 mM acetic acid in pH 7 phosphate buffer in a 0 to +1.5 V (vs. SCE) potential range at a scan rate of 50 mV s^{-1} but no peak corresponding to acetic acid oxidation was observed. In order to exclude possible interference of other metabolites present in the real samples, a separation of oxalate from real sample matrix is desirable prior to oxalate determination.

Next the response of the BPPG electrode to different concentrations of oxalate has been examined. The study was performed in 0.1 M K_2SO_4 at scan rate of 50 mV s^{-1} with concentration of oxalate between 0.5 and 3.5 mM (Fig. 3A). The peak oxidation current was found to linearly increase with increased oxalate concentration. Limit of detection for oxalate using BPPG electrode was determined based on the 3 sigma method [42] using linear sweep voltammetry data. Blank solution was spiked with 2 μM additions up to 30 μM oxalate concentration and peak current monitored and plotted against oxalate concentration (Fig. 3B). From this graph a limit of detection was evaluated using 3 sigma method:

$$LOD = 3\delta/b \quad (2)$$

where δ is the standard deviation of the y-coordinates from the line of best fit and b the slope of the same line. The limit of detection for oxalate using BPPG electrode was calculated to be 0.7 μM. The standard addition procedure for the determination of the detection limit of oxalate by the BPPG electrode (with 2 μM additions between 2 to 30 μM) in 0.1 M K_2SO_4 was repeated 10 times giving a standard deviation of less than 3%.

The value obtained is comparable or lower than values obtained by enzymatic biosensors (Table 1). The advantages of BPPG electrode for oxalate determination are that it is

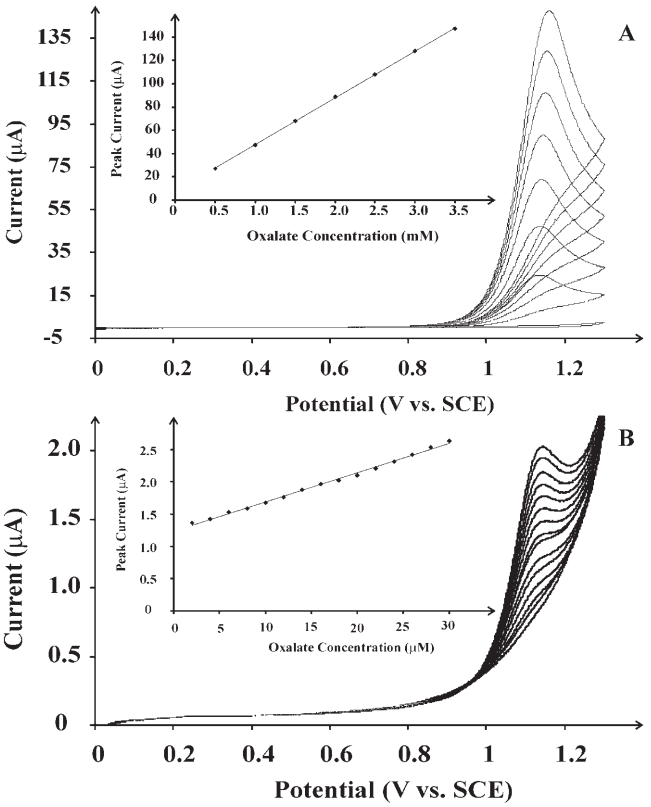

Fig. 3. Cyclic voltammograms of BPPG electrode in 0.1 M K_2SO_4 with increasing oxalate concentration from 0 to 3.5 mM at scan rate of 50 mV s^{-1} (A) and linear sweep voltammograms of BPPG electrode in 0.1 M K_2SO_4 with increasing oxalate concentration from 0 to 30 μM at scan rate of 50 mV s^{-1} with standard addition plot for 2 μM additions (B).

Table 1. Comparison of performances of different sensors for oxalates. OXO: oxalate oxidase; CAT: catalase; OXD: oxalate decarboxylase; PB: Prussian Blue; HRP: horseradish peroxidase; TB: Toluidine Blue; CPE: carbon paste electrode.

Electrode	*LOD* (μM)	Linear range (μM)	Sensitivity (A M^{-1} cm^{-2})
OXO/CrHCF-graphite electrode [21]		2.5 – 400	0.031
OXO/preactivated membrane [43]	10 collagen 0.04 Nylon		
spinach tissue homogenate/Clarck-type oxygen electrode [22]	10	10 – 100	
acrylamide gel – OXD [44]	10	0 – 200	
OXO/PB/self-doped polyaniline film [23]	80	80 – 450	0.1313
OXO/HRP TiO_2/TB – CPE [24]	90	100 – 2000	
Pd nanoparticles modified CPE [28]	20	20 – 10000	
OXD/CO_2-sensing electrode [45]	40	200 – 10000	
Ru(II) complex/Nafion-modified Pt gauze electrode [46]	50	100 – 5000	

simple to construct and does not require any expensive chemicals, enzymes and apparatus. Further work would have to be performed before the method could be applied for the determination of oxalate in real samples (e.g., biological fluids). In particular extensive studies of sensitivity and reproducibility as well as testing of possible multiple interferences in these specific media would be needed.

4. Conclusions

The determination of oxalate ions is of great significance in food industry and clinical analysis. In the present paper the electroactivity of different carbon based electrodes: BPPG, EPPG and GC electrode, for the determination of oxalate ions has been studied. A characteristic peak for oxalate oxidation was obtained at all three electrodes: BPPG, EPPG and GC electrode. The BPPG electrode showed superior behavior and significant advantages for oxalate oxidation in comparison with EPPG and GC electrode. The BPPG electrode demonstrated good performance for the detection of oxalate ions over wide range of pHs and concentrations with a sensitivity of 0.039 AM^{-1} and a detection limit of oxalate of 0.7 μM. The value of the limit of detection obtained with BPPG electrode is comparable or lower than those obtained by enzymatic biosensors; moreover, such a low detection limit has been attained without use of expensive chemicals and complicated procedures.

5. Acknowledgements

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6. References

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