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(Bio)sensors based on manganese dioxide-modified carbon substrates: retrospections, further improvements and applications☆

Negussie W. Beyene[a,1], Petr Kotzian[b], Klemens Schachl[a], Hailemichael Alemu[c], Emir Turkušić[d], Amira Čopra[d], Helmut Moderegger[a], Ivan Švancara[b], Karel Vytřas[b,*], Kurt Kalcher[a,2]

[a] *Institute of Chemistry-Analytical Chemistry, Karl-Franzens University, A-8010 Graz, Austria*
[b] *Department of Analytical Chemistry, University of Pardubice, CZ-532 10 Pardubice, Czech Republic*
[c] *Department of Chemistry, National University of Lesotho, P.O. Roma 180, Lesotho*
[d] *Department of Chemistry, University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina*

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Abstract

An overview is presented which summarizes our accomplishment in the development of sensors and biosensors based on heterogenous carbon electrodes modified with manganese dioxide. Brief account of each sensor and biosensor has been given and example of real sample applications provided where appropriate.

Keywords: Carbon paste electrodes; Screen-printed electrodes; Biosensors; Modification; Electroanalysis

1. Introduction

Heterogeneous carbon materials have been used as electrochemical sensors and biosensors because of their availability in a variety of forms, low cost, broad exploitable potential window, low background current, chemical inertness, ease of chemical derivatization and modification, and suitability for various applications [1,2]. Among the various carbon-based electrodes available for the development of electrochemical sensors and biosensors, carbon paste electrodes (CPEs) and screen-printed carbon electrodes (SPCEs) have got widespread popularity due to their ease of preparation and modification, ease of surface renewal and reproducibility in case of CPEs, and mass production of highly reproducible electrodes in case of SPCEs [2–4].

One of the analytes detected at CPEs and SPCEs is hydrogen peroxide because of its environmental, biological and industrial importance. For this purpose, amperometric and voltammetric methods have been used. However, its direct oxidation or reduction at bare electrodes causes a problem in analytical applications because of the high overvoltage required. These phenomena can be controlled by deliberately attaching chemical reagents to the electrode surface so that manipulating the nature of the surface is possible. Hence, the name *chemically modified electrodes* was coined [5–8]. Heterogeneous carbon electrodes were among the first to be chemically modified.

Modified electrodes are appreciated for their main advantages such as, reduction of the H_2O_2 overvoltage and hence diminishing the interference from other species by promotion of electron transfer reactions which may increase the selectivity, specificity and reproducibility of the electrode surface, and improving the detection limit [1–8]. These electrodes can be prepared by chemisorption, covalent bond formation between specific functional group on the electrode surface and the reagent, coating the electrode with polymeric

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* Corresponding author. Tel.: +420 466 037 712; fax: +420 466 037 068.
E-mail addresses: karel.vytras@upce.cz (K. Vytřas), kurt.kalcher@uni-graz.at (K. Kalcher).
[1] Current address: Department of Chemistry, University of Pretoria, 0002 Pretoria, South Africa.
[2] Tel.: +43 316 380 5310; fax: +43 316 380 9845.

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films (including electropolymerization of monomers) and forming heterogeneous layers (e.g., thorough mixing of the modifier with carbon paste or carbon ink) [1,2]. Among these, the simplest, most reliable and frequently used modification technique is admixing the modifier with conducting pastes and inks prior to putting the electrode in the desired geometry. In this context, numerous transition metal species (oxides and complexes) have been used as modifiers. Ferrocene derivatives, organometallics such as ruthenium and osmium complexes, hexacyanoferrate, Methylene Blue and Methyl Viologen are most often employed [8–11]. Several excellent reviews dealing with chemically modified electrodes [12–15], mediated biosensors [16,17] and modified CPEs [3,18] are available. A very recent example is the use of manganese dioxide. Since the mid-1990s, our group has been actively engaged in the development of sensors and biosensors for several analytes based on heterogeneous carbon electrodes modified with manganese dioxide. In the first phase of the investigation, a comparison was made between the performances of the sensors produced by film- and bulk modification of CPEs and SPCEs. In the second phase, application of these electrodes as sensors for H_2O_2, ascorbic acid and uric acid as well as incorporation of bioactive components for fabrication of biosensors has been assessed [19]. The present review focuses entirely on MnO_2-modified sensors and biosensors that have been developed by our group and collaborators since the launching of this research in the mid-1990s.

2. Bulk modification

2.1. MnO_2 bulk-modified CPEs

In our first report [20] it has been demonstrated that the cyclic voltammograms (CVs) of plain and MnO_2 bulk-modified CPE are entirely different (Fig. 1). A reduction peak at around −1200 mV (curve a) with the unmodified electrode has been assigned to the reduction of hydrogen peroxide overlapped with the reduction of oxygen-containing groups of the carbon particles. Another reduction peak from −200 to −800 mV has been observed at the modified electrode in the absence of hydrogen peroxide (curve c). This has been attributed to the overall reduction of MnO_2 to oxides of lower oxidation states (Mn^{II} and Mn^{III}). An oxidation peak above potential value (−200 mV) appeared due to re-oxidation of Mn^{II} and Mn^{III} to MnO_2 forming a thin film of the MnO_2 upon cycling. The CV of the modified electrode in the presence of H_2O_2 (curve d) shows a reduction peak at −1000 mV that was due to the reduction of MnO_2 and/or H_2O_2 overlapped with reduction of the carboxyl group of the carbon paste. Oxidation occurred again above −300 mV due to the retransformation of lower oxidation state manganese oxides to MnO_2. However, the distinct signal between 400 and 500 mV indicated that oxidation to MnO_2 increased significantly in the presence

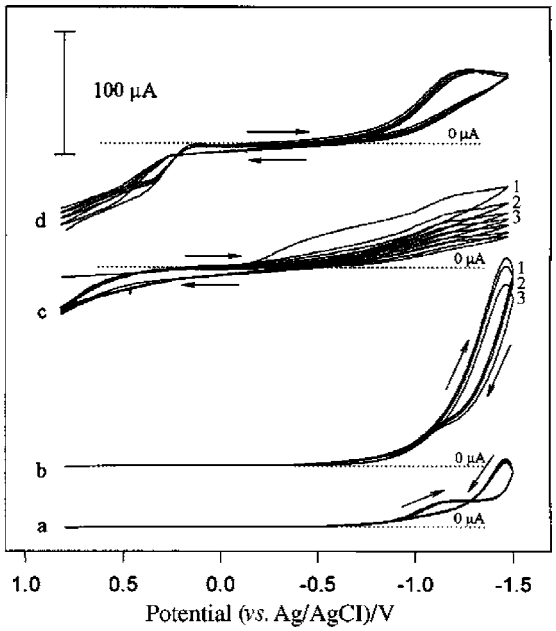
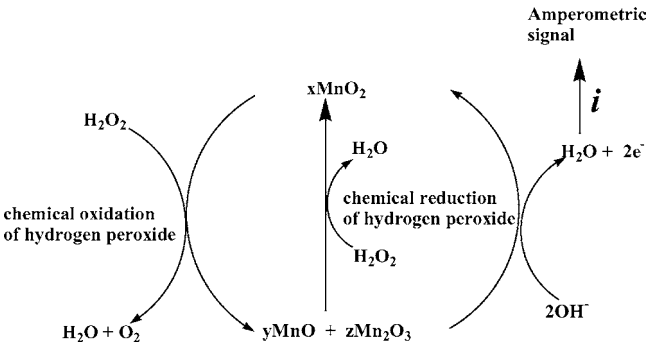


Fig. 1. Cyclic voltammograms of hydrogen peroxide at an unmodified (a, b) and an MnO_2-modified CPE (c, d). Equilibration time, 30 s; initial potential, 0.8 V; final potential, −1.5 V vs. Ag/AgCl; scan rate, 20 mV s^{-1}; supporting electrolyte, 0.2 M NH_3–NH_4Cl buffer; H_2O_2 concentration, 0 (a, c) and 50 mg l^{-1} (b, d) [20]. Reproduced by permission of the Royal Society of Chemistry.

of H_2O_2. Thus, in the potential range of 400–500 mV, the modifier has mediating activity towards H_2O_2 that lead us to the mechanism depicted in Scheme 1.

Maximum signal in the CV has been obtained when the MnO_2 concentration in the paste was in the range of 3.8–5.7% (m/m). The modified electrode has been used to determine H_2O_2 in a flow injection (FI) amperometric mode using ammonia–ammonium chloride buffer (pH 9.5) as a carrier. Figs. 2 and 3 show a FI amperogram obtained using the modified CPE. With an applied potential of 460 mV versus Ag/AgCl, an injection volume of 50 µl, and a flow rate of 0.9 ml min^{-1}, the sensor exhibited a detection limit of 45 µg l^{-1} and a linear concentration dependence up to 350 mg l^{-1}. It was used to determine H_2O_2 in hair blonding boosters and mouthwashes giving excellent correlation with



Scheme 1. Catalytic redox cycle of manganese dioxide and chemical oxidation of H_2O_2 [21].

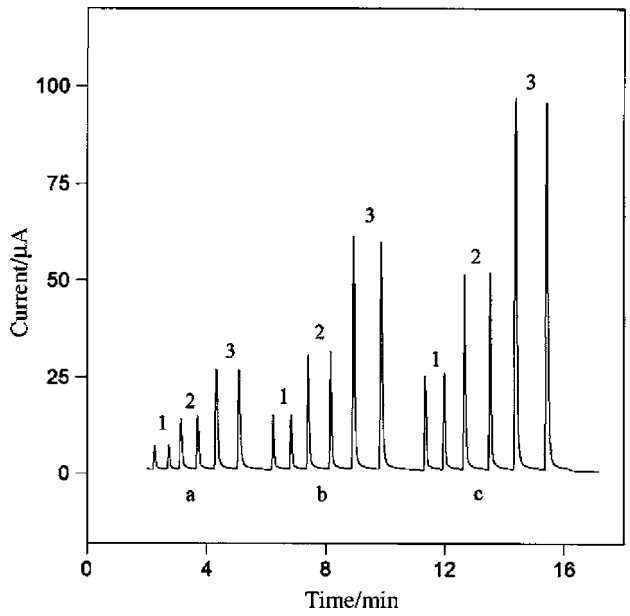


Fig. 2. FI amperometric response for hydrogen peroxide. Applied potential, 460 mV vs. Ag/AgCl; carrier, 0.2 M NH_3–NH_4Cl buffer; flow rate, 0.9 ml min^{-1}; hydrogen peroxide concentration (a) 20, (b) 40 and (c) 60 mg l^{-1}; injection volumes (1) 25, (2) 50 and (3) 100 μl [20]. Reproduced by permission of the Royal Society of Chemistry.

the result obtained by iodometric titration. A wide range of cations and anions were studied for their interference in the amperometric determination of H_2O_2. Cadmium, iron and lead among the cations; tartarate, borate, hypochlorite and vanadate among the anions; as well as ascorbic acid and paracetamol were found to interfere [22].

A glucose biosensor based on MnO_2 and glucose oxidase (Gox) in a double-bulk-modified CPE has been described [23]. It contained 3.8% (m/m) of both MnO_2 and GOx in the paste. The performance of the biosensor was tested in both hydrodynamic and FI mode. In FI and at an applied potential of 480 mV versus Ag/AgCl and a flow rate of 0.2 ml min^{-1} of the carrier (0.2 M phosphate buffer, pH 7.5), the biosensor exhibited a linear concentration dependence in the range between 20 and 500 mg l^{-1} glucose and a detection limit of 1.3 mg l^{-1}. Its applicability has been demonstrated by using it for the determination of glucose in wines. The results were in very good agreement with those of the high performance liquid chromatography (HPLC). Detailed studies revealed that ascorbic acid and uric acid still interfered. However, on the other hand, this effect was exploited to develop a sensor for determination of ascorbic acid in pharmaceutical preparations [24]. A concentration dependence of such MnO_2 bulk-modified CPE showed linearity from 50 to 250 mg l^{-1} and a detection limit of 0.13 mg l^{-1} (pH 5.0 phosphate buffer as supporting electrolyte). The proposed reaction mechanism is shown in Scheme 2.

2.2. MnO_2 bulk-modified SPCEs

Screen-printing technology is well known for its advantages in producing reproducibly inexpensive, disposable sensors and biosensors. To make use of these advantages, carbon ink was bulk modified with MnO_2 and then printed on a ceramic support [25]. The sensor exhibited enhanced analytical performance compared to the corresponding carbon paste based sensor. Accordingly, the carbon ink was

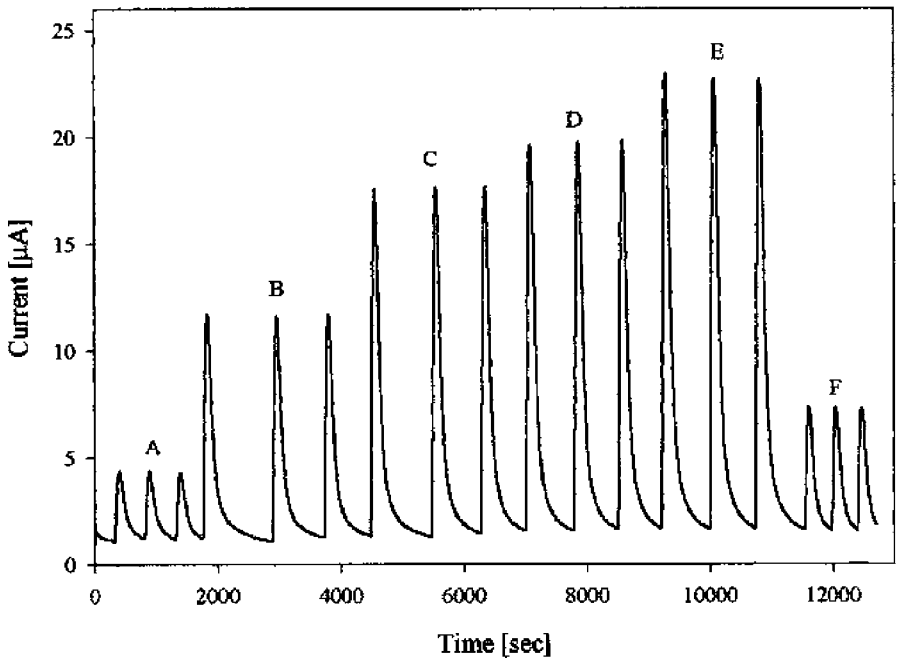
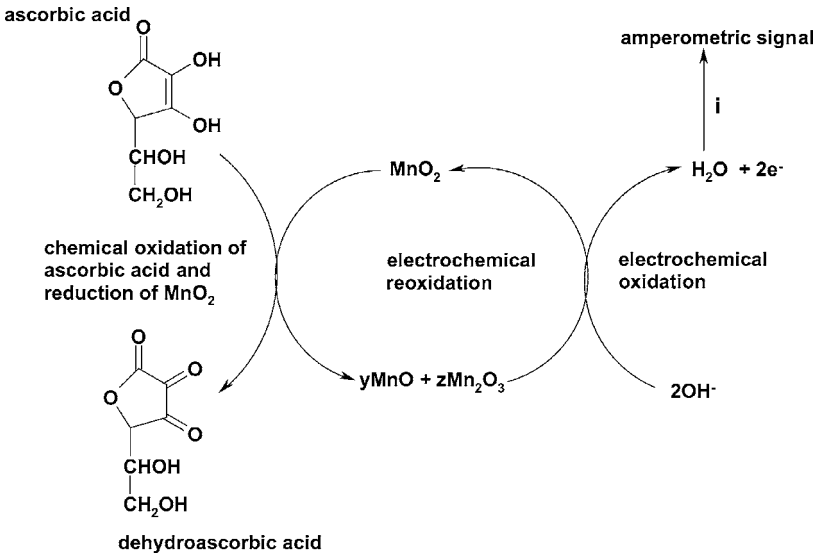


Fig. 3. Amperometric FI response of glucose with an MnO_2–GOx double-bulk-modified SPCE. Applied potential, 480 mV vs. Ag/AgCl; flow rate, 0.2 ml min^{-1}; carrier, phosphate buffer pH 7.5 (0.1 mol l^{-1}); injection volume, 250 μl; concentration of glucose (A) 500, (B) 2000, (C) 3500, (D) 4000, (E) 5000 and (F) 1000 mg l^{-1}. Reprinted from reference [25] by courtesy of Marcel Dekker Inc.



Scheme 2. Proposed reaction mechanism of ascorbic acid at MnO_2 bulk-modified carbon electrodes [24].

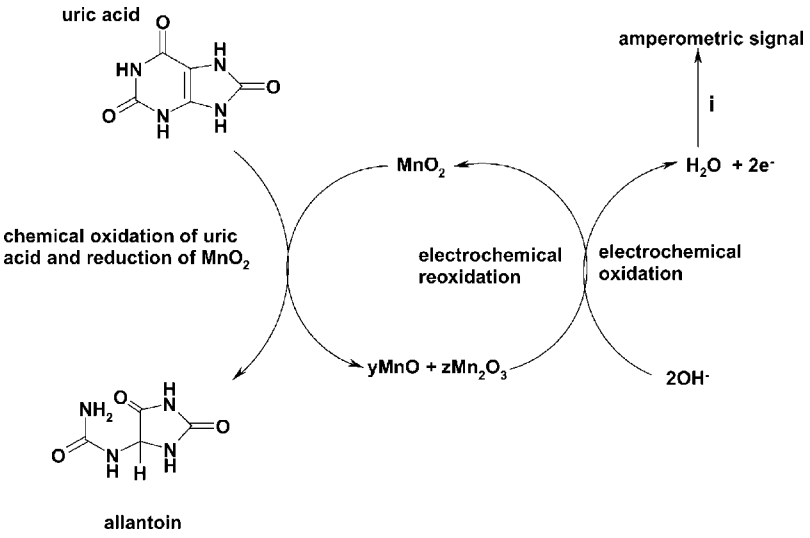
double-bulk-modified with 5% (m/m) MnO_2 and GOx each prior to printing. Again, the biosensor performance was tested in both hydrodynamic and FI modes (the second presented in Fig. 3).

In FI mode, at 480 mV versus Ag/AgCl and a flow rate of 0.2 ml min^{-1} of the carrier (0.1 M phosphate buffer, pH 7.5), the biosensor exhibited a linear response in the concentration range between 2 and 2500 mg l^{-1} glucose with a detection limit of 85 μg l^{-1}. The applicability of the biosensor was demonstrated by determining glucose in beer and wine samples, the results were in good agreement with a commercial glucose kit. It has also been successfully used to determine bonded glucose in cellobiose, saccharose, and (−)-4-nitrophenyl-β-D-glucopyranoside [26]. The bonded glucose was broken down by off-line pre-treatment using the enzyme glucosidase. Interference studies showed significant interfering influence from ascorbic and uric acids again. This behaviour was utilized to produce both uric acid and ascorbic acid sensors based on MnO_2 bulk-modified SPCEs. The response of the ascorbic acid sensor was linear in the range between 50 and 250 mg l^{-1} with a detection limit of 0.2 mg l^{-1} when used in a hydrodynamic amperometric mode (applied potential of 600 mV versus Ag/AgCl, pH 5.0 of the 0.05 M supporting electrolyte). This sensor was used to determine ascorbic acid in tablets [27]. Likewise, the uric acid sensor was used in a FI system and best signals were recorded at an applied potential of 540 mV versus Ag/AgCl with a flow rate of 0.2 ml min^{-1} of the carrier (0.05 M phosphate buffer, pH 7.5). A linear relation between signal and concentration was obtained in the range of 10–120 mg l^{-1} with a detection limit of 1.0 mg l^{-1}. The sensor was used to determine uric acid in human urine with off-line pre-treatment that employed ascorbate oxidase to remove any interfering ascorbic acid [28,29]. The proposed reaction mechanism for uric acid determination is shown in Scheme 3.

Having continued in our effort, we tried to produce glutamate biosensors based on MnO_2 and glutamate oxidase (GlOx) double-bulk-modified SPCEs [26]. As a result of the high price of the enzyme, we used very small amounts of GlOx for bulk modification as compared to that of GOx (5% mass ratio) in case of the glucose biosensor. Thus, in further experiments, we sought for an economical and simple immobilization technique that could be compatible with such a sensor.

As an alternative to bulk modification, we studied the immobilization of oxidases with Nafion® films taking GOx as a model enzyme [30]. The Nafion®-enzyme film was formed by drop-coating the suspension onto the surface of the electrode. Comparisons were made among biosensors developed by using as received, neutralized, and diluted (to 1% in methanol, ethanol or phosphate buffer) Nafion® in a FI amperometric mode [31]. The amperometric response to injections of standard glucose solution was the highest for biosensors produced using neutralized Nafion® (Fig. 4). The enzyme load was also investigated, an amount of 50 μg per electrode was found to give good signals along with favourable economy. Three different biosensors were prepared and tested for immobilization repeatability by injecting standard glucose solution. A relative standard deviation of 5.7% was recorded as the mean from the three biosensors. After the operational parameters had been optimised, the biosensor exhibited linear concentration dependence in the range of 10–750 mg l^{-1} and a detection limit of 0.85 mg l^{-1} glucose (applied potential 440 mV versus Ag/AgCl, flow rate 0.2 ml min^{-1}, see Fig. 5). Preliminary investigation on incorporation of a hydrogen peroxide permselective membrane layer (to eliminate interference from ascorbic acid and uric acid) prior to enzyme immobilization gave a promising result with polyurethane, especially for single-shot biosensors.

The above mentioned immobilization procedure was then used to immobilize GlOx, the resulting glutamate biosensor



Scheme 3. Proposed reaction mechanism for oxidation of uric acid on MnO_2 bulk-modified carbon electrodes [29].

was used in a FI system [32]. Typical responses for different concentrations of glutamate are shown in Fig. 7. With an applied potential of 440 mV versus Ag/AgCl, a flow rate of 0.2 ml min^{-1} and a pH 7.5 of the carrier (0.1 M phosphate buffer), it showed a linear relationship of the current response on the glutamate concentration in the range of 10–160 mg l^{-1}, and a detection limit of 1.7 mg l^{-1}. The biosensor was used to determine monosodium glutamate in food seasonings; the values obtained were in good agreement with those obtained spectrophotometrically. Furthermore, the glutamate biosensor exhibited extraordinary stability when left in the FI system at a flow rate of 0.1 ml min^{-1} at room temperature retaining 50% of the original response towards glutamate even after 65 days. Stored in the working buffer for more than 60 days, the same biosensor showed extended linear range. The long-term stability was attributed to the effect of MnO_2 on GlOx (similarly to a report on D-amino acid oxidase [33]).

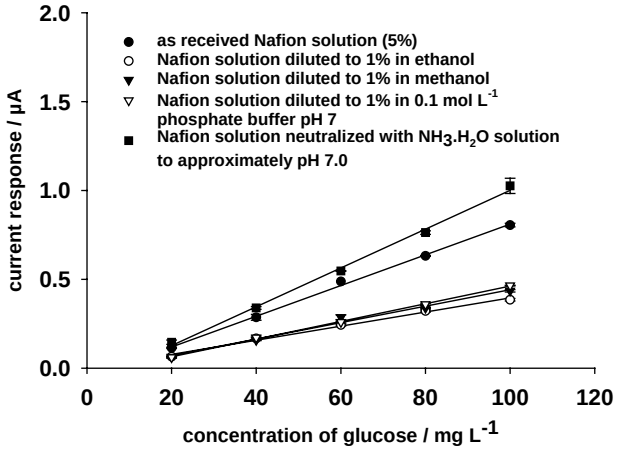


Fig. 4. Comparison of amperometric responses of biosensors produced by enzyme castings using diluted (to 1% in different solvents), neutralized and as received (5%) Nafion® solutions. The final enzyme load in each case was 95.2 μg in two subsequent layers. Working conditions: applied potential, 400 mV vs. Ag/AgCl (3 mol l^{-1} KCl) reference electrode; flow rate, 0.2 ml min^{-1}; injection volume, 100 μl; carrier, phosphate buffer (0.1 mol l^{-1}, pH 7.5). Measurements were done in triplicates [31]. Reproduced by permission of the South African Chemical Institute.

Neurolathyrism is a crippling disease caused by excessive and prolonged consumption of meals solely made from grass pea (*Lathyrus sativus*) seeds. The causative agent is believed to be the non-essential free amino acid β-*N*-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) present in the seeds. It is also known that GlOx has some activity towards β-ODAP with lower kinetics than it shows towards the main substrate, glutamate [34]. For that reason, we tried to employ the GlOx-based biosensor in assaying the toxin. Consequently, the glutamate oxidase electrode developed was tested for its responses to injections of standard β-ODAP solutions at a lower flow rate (0.1 ml min^{-1}). Having used the aforementioned parameters for glutamate, the biosensor exhibited a linear concentration dependence in the range of 50–500 mg l^{-1} with a detection limit of 29 mg l^{-1} [35]. According to our knowledge, this was the first attempt on a β-ODAP biosensor based on SPCEs [36]. To avoid interference from glutamate present in grass pea seeds, an off-line sample pre-treatment procedure with glutamate decarboxylase was used that decarboxylates glutamate but leaves β-ODAP unaffected. The β-ODAP biosensor was used to determine the content of the toxin in grass pea seed samples [35–37].

More recently, a sarcosine biosensor was developed by immobilizing sarcosine oxidase via Nafion® film entrapment. The sarcosine biosensor was used at an applied potential of 380 mV versus Ag/AgCl, a flow rate of 0.2 ml min^{-1} in a carrier (0.1 M phosphate buffer, pH 8.25). The biosensor exhibited a linear increase of the amperometric signal with the sarcosine concentration in the range of 10–250 mg l^{-1} with a detection limit of 2.5 mg l^{-1}. The sarcosine biosensor

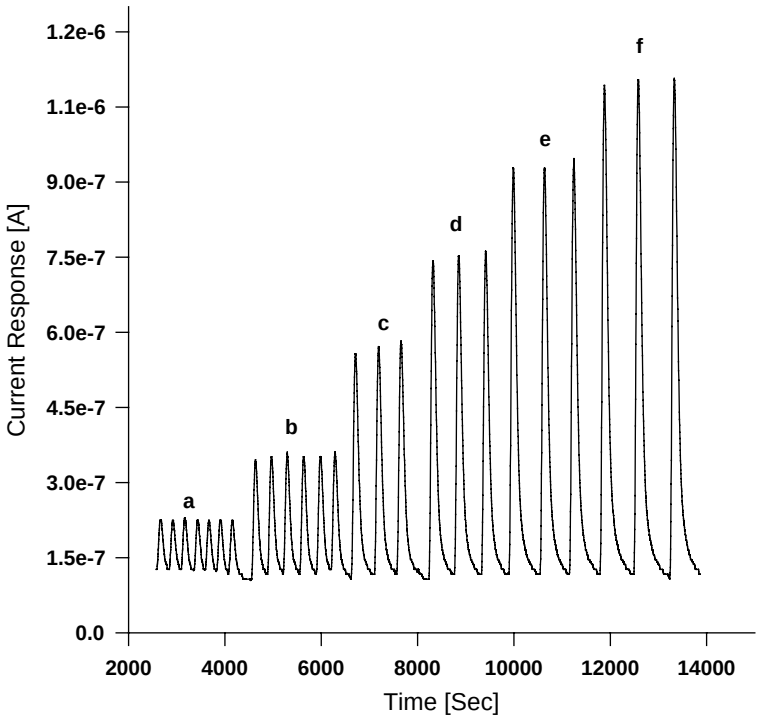


Fig. 5. Typical response of a glutamate biosensor developed for concentrations of (a) 10, (b) 20, (c) 40, (d) 60, (e) 80 and (f) 100 mg l^{-1} glutamate at an applied potential of 440 mV vs. Ag/AgCl; flow rate, 0.2 ml min^{-1}; carrier, 0.1 mol l^{-1} (pH 7.75) phosphate buffer [32]. Reproduced by permission of the South African Chemical Institute.

had a good selectivity over other biogenic amines and it was applied in food analysis. The data seemed very promising, especially for determining the freshness of fish and other foodstuffs [38].

3. Film modification

3.1. MnO_2 film-modified CPEs

A MnO_2 film has been formed by electrolysis of ammonia/ammonium chloride buffer solution (pH 9.5) containing 20 mg l^{-1} manganese (as chloride) at an applied potential of 600 mV for 60 min [39]. CVs of hydrogen peroxide at a plain and at an MnO_2 film-modified CPE were run (Fig. 6). In the presence of H_2O_2, the unmodified CPE showed a reduction peak starting at −1.20 V (towards the cathodic scan). This signal was overlapped with that of the reduction of oxygen-containing groups of the carbon paste (curve b).

In the absence of H_2O_2, the MnO_2 film-modified CPE showed a reduction signal starting at +300 mV and increased significantly below −800 mV (in the cathodic scan). The signal was attributed to formation of lower oxidation state manganese oxides (MnO and Mn_2O_3). Above 400 mV, re-oxidation of these oxides to MnO_2 occurred (curve c). The CV of the MnO_2 film-modified CPE in the presence of H_2O_2 had a different pattern (curve d). The reduction signal that starts at −700 mV was due to the reduction of MnO_2 that had been enhanced by the catalytic reduction of H_2O_2. Oxidation occurred above +300 mV that showed significant increase with a maximum at around +500 mV attributed to the presence of H_2O_2 (oxidation of H_2O_2 and reduction of MnO_2). The shape of the CV in the anodic scans, i.e., above a potential of +300 mV, clearly indicated the catalytic

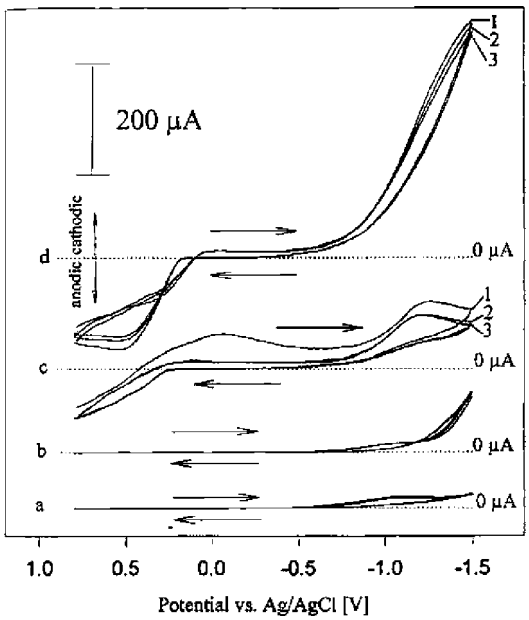


Fig. 6. Cyclic voltammograms of hydrogen peroxide at an unmodified (a, b) and a MnO_2 film-modified CPE (c, d). Equilibration time, 30 s; initial potential, 0.8 V; final potential, −1.5 V vs. Ag/AgCl; scan rate, 20 mV s^{-1}; supporting electrolyte, NH_3/NH_4Cl buffer (0.2 mol l^{-1}); H_2O_2 concentration, 0 mg l^{-1} (a, c) and 50 mg l^{-1} (b, d). Reprinted from reference [39] by courtesy of Marcel Dekker Inc.

(mediating) activity of the modifier. As compared to that of the MnO_2 bulk-modified CPE, the reduction current at the film-modified CPE was very high. Apparently, the catalytic activity of the MnO_2 film was more effective than that of MnO_2 in the bulk of the electrode. For production of disposable sensors and biosensors, however, the latter was favoured due to its experimental convenience. The film-modified CPE sensor was used in a flow system for determination of H_2O_2 in rainwater. With an applied potential of 460 mV versus Ag/AgCl, a flow rate of 1 ml min^{-1} of the carrier (0.2 M NH_3/NH_4Cl buffer, pH 9.5) and an injection volume of 50 μl, the sensor showed linear response towards H_2O_2 concentration from 5 to 450 mg l^{-1} with a detection limit of 4.7 μg l^{-1}. As usually, notable interference was observed from ascorbic acid and uric acid again.

3.2. MnO_2 film-modified SPCEs

In these sensors, MnO_2 was electrodeposited onto the surface of the plain SPCE by applying a potential of +600 mV for 120 s using a pH 9.5 ammonia buffer that contained 10 mg l^{-1} Mn^{2+} [40]. The MnO_2 film-modified strip was rinsed with water and dried at 60 °C for 60 min. Linear sweep voltammogramms (LSVs) of H_2O_2 at plain and modified electrodes in two different 0.1 M phosphate buffers (pH 7.5 or 9.5) were recorded (Fig. 7). In the presence of H_2O_2, plain SPCEs showed increasing oxidation current with increasing positive potential at both pH values (curves a and e). This pattern was not observed in film-modified CPE and thus the oxidation might be due to the matrix components in the carbon ink. Additions of H_2O_2 did not make any notable change in the LSVs (curves b and f). In the absence of H_2O_2, the film-modified SPCEs yielded very small background currents as compared to the plain SPCEs (curves c and g); additions of H_2O_2 produced oxidation waves (Ia). The oxidation signal started at about 450 mV at pH 7.5 (curve d) but at lower potential at pH 9.5 (curve h). Moreover, an increase in the height of the signal was observed at pH 9.5. Like in the case of CPEs (with the same mechanism depicted above), the MnO_2 film on SPCEs acted as a mediator for the oxidation of H_2O_2. The sensor was used for the determination of H_2O_2 in rainwater in a FI system. A typical record for series of injections of different concentrations is presented in Fig. 8.

With an applied potential of 480 mV versus Ag/AgCl, a flow rate of 0.6 ml min^{-1} of the carrier (0.1 M phosphate buffer, pH 7.5), and an injection volume of 250 μl, the sensor exhibited linear concentration dependence in the range of 5–10 000 μg l^{-1} and a detection limit of 2.3 μg l^{-1}. As could be expected, significant interference was observed again from both ascorbic and uric acids. Other than the aforementioned, advantages of film-modified SPCEs over the film-modified CPEs are the shorter plating time (2 min versus 60 min), the time required for analyses, better stability and repeatability.

Comparison of bulk modification with film modification reveals that the latter has advantages of lower detection limit

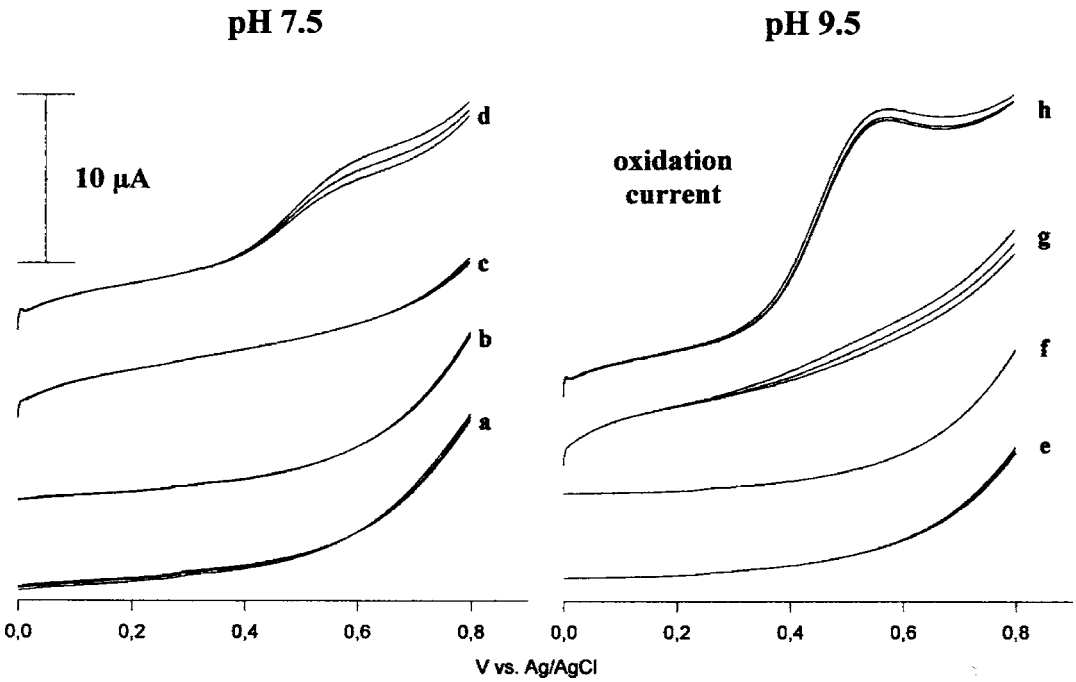


Fig. 7. Linear sweep voltammogramms at an unmodified (a, b, e, f) and a MnO_2 film-modified SPE (c, d, g, h) at pH 7.5 and at pH 9.5. Equilibration time, 15 s; initial potential, 0.8 V vs. Ag/AgCl; scan rate, 20 mV/s; supporting electrolyte, 0.1 mol l^{-1} phosphate buffer, pH 7.5 (a–d) or pH 9.5 (e–h); H_2O_2 concentrations, 0 mg l^{-1} (a, b, e, f) or 10 mg l^{-1} (c, d, g, h); three repetitive scans for each curve Reproduced by permission of Springer-Verlag GmbH from reference [40].

Table 1
Sensors and biosensor based on MnO_2-modifed carbon electrodes, optimised parameters and figures of merit produced

Analyte	Transducer	Applied potential (mV)	Flow rate ($ml\,min^{-1}$)	pH	Linear range ($mg\,l^{-1}$)	Detection limit (3σ) ($\mu g\,l^{-1}$)	Sample	Reference
H_2O_2	CPE/MnO_2-b	460	0.9	9.50	0.5–350	45	Hair spray and mouth wash	[20]
H_2O_2	CPE/MnO_2-f	460	1.0	9.50	0.005–450	4.7	Rain water	[39]
H_2O_2	SPCE/MnO_2-f	480	0.6	7.50	0.005–10	2.3	Rain water	[40]
Ascorbic acid	SPCE/MnO_2-b	600	–[a]	5.00	50–250	200	Pharmaceutical tablets	[27]
Ascorbic acid	CPE/MnO_2-b	600	–[a]	5.00	50–250	130	Pharmaceutical tablets	[24]
Uric acid	SPCE/MnO_2-b	540	0.2	7.50	10–120	990	Urine	[28,29]
Glucose	SPCE/MnO_2-b/GOx-b	480	0.2	7.50	2–2500	85	White wine and beer	[25]
Glucose	CPE/MnO_2-b/GOx-b	480	0.2	7.50	20–500	1300	White wine	[23]
Glucose	SPCE/MnO_2-b/GOx-Nf	400	0.2	7.50	10–750	850	N/A	[30,31]
Glutamate	SPCE/MnO_2-b/GlOx-Nf	440	0.2	7.75	10–160	1700	Food seasonings	[32]
β-ODAP	SPCE/MnO_2-b/GlOx-Nf	440	0.1	7.75	50–500	29000	Grass pea seeds	[35,36]
Sarcosine	SPCE/MnO_2-b/SOx-Nf	380	0.2	8.25	10–250	25000	Fish, cheese, meat	[38]

b: bulk; f: film; Nf: Nafion film.
[a] Hydrodynamic amperometry.

and wider dynamic range due to the high probability of chemical decomposition of the H_2O_2 at the surface of the electrodes, i.e., a high ratio of H_2O_2 to catalytically active MnO_2. The main disadvantage of film-modified electrodes is that they require frequent film formation where as the bulk-modified electrodes can work for weeks without significant change in the response. Thus, bulk-modified electrodes are preferred to film-modified ones for production of disposable one-shot sensors and biosensors. Table 1 summarizes the sensors and biosensors with the corresponding parameters used to characterize their performance as well as the analytical figures of merit obtained.

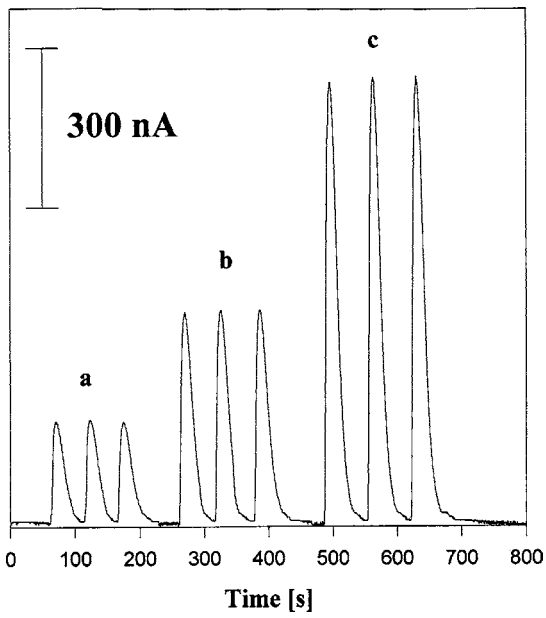


Fig. 8. Amperometric FI response of hydrogen peroxide with an MnO_2 film-modified SPCE. Applied potential, 480 mV vs. Ag/AgCl; carrier, phosphate buffer pH 7.5 ($0.1\,mol\,l^{-1}$); flow rate, $0.6\,ml\,min^{-1}$; injection volume, 250 μl; hydrogen peroxide concentrations (a) 50, (b) 100 and (c) $200\,\mu g\,l^{-1}$. Reproduced by permission of Springer-Verlag GmbH from reference [40].

4. Conclusions

In general, MnO_2-modified heterogeneous carbon electrodes described here are very simple to prepare. Additionally, they are cheap and the modifier is non-toxic. Unlike other modifiers (Prussian Blue [41], ferrocene-polyaniline [42], Cu-heptacyanonitrosylferrate [43], etc.) that can be affected by other peroxic species (e.g., persulphate) and oxygen, the MnO_2-modified electrodes are unaffected by them. A paper by Taha and Wang [44] should be mentioned in which a MnO_2 film-modified glassy carbon electrode was found inappropriate for biosensor applications in strongly alkaline media. The MnO_2-modified electrodes discussed here were applied satisfactorily in both neutral and slightly alkaline media assuring their compatibility with oxidase catalysed reactions and hence suitability for biosensor applications. A very recent report by Dousikou et al. re-confirmed the suitability of MnO_2-modified carbon electrodes for analysis at neutral pH as well as their superiority, in terms of sensitivity, over platinum-modified carbon electrodes [45]. That is why Roche Diagnostics is going to commercialise electrochemical thick film biosensors based on SPCE that incorporates MnO_2 in the carbon ink prior to the printing step for glucose, lactate, urea, and creatinine [46]. Concerning further improvements, one can state that there are still many things to be studied. For example, a search for another modifiers acting similarly to MnO_2 may also be an object of interest [45,47].

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