Full Paper

Dopamine and Glucose Sensors Based on Glassy Carbon Electrodes Modified with Melanic Polymers

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Abstract

This work deals with the study of polymers electrogenerated from different catechols at glassy carbon electrodes and the analytical applications of the resulting modified electrodes for dopamine quantification and glucose biosensing. The electropolymerization was performed from a 3.0×10^{-3} M catechol solution (catechol, dopamine, norepinephrine, epinephrine or L-dopa in a 0.050 M phosphate buffer pH 7.40) by applying 1.00 V for 60 min. The properties of the polymers are very dependent on the nature of the catechol, L-dopa being the best. Glassy carbon electrodes modified with melanic polymers electrogenerated from L-dopa and norepinephrine were found to be suitable for dopamine determinations in flow systems, although the behavior was highly dependent on the nature of the monomer. Detection limits of 5.0 nM dopamine and interferences of 9.0 and 2.6% for 5.0×10^{-4} M ascorbic acid and 5.0×10^{-5} M dopac, respectively, were obtained at the glassy carbon electrode modified with a melanin-type polymer generated from L-dopa (using 1.0×10^{-3} M AA in the measurement solution). The advantages of using a melanin-type polymer generated from dopamine to improve the selectivity of glucose biosensors based on carbon paste electrodes containing Pt and glucose oxidase (GOx) are also discussed. The resulting bioelectrodes combines the high sensitivity of metallized electrodes with the selectivity given by the polymeric layer. They exhibit excellent performance for glucose with a rapid response (around 10 seconds per sample), a wide linear range (up to 2.5×10^{-2} M glucose), low detection limits (143 μM) and a highly reproducible response (R.S.D of 4.9%). The bioelectrodes are highly stable and almost free from the interference of large excess of easily oxidizable compounds found in biological fluids, such as ascorbic acid (AA), uric acid (UA) and acetaminophen.

Keywords: Polymer, Melanin, Flow injection, Glassy carbon, Dopamine, Dopac, Ascorbic acid, Glucose biosensor, Metallized electrode, Platinum

1. Introduction

Polymers have been largely used in the development of biosensors [1, 2]. The permselective properties of many polymers towards negatively charged compounds, have allowed their use as a barrier in the detection of different analytes. The efficiency of some polymeric films to effectively reject the negatively charged ascorbic acid (AA) and 3,4-dihydroxyphenylacetic acid (dopac) has been widely demonstrated [3–8]. Yuan et al. [3] have reported on the use of a poly(2-picolinic acid) modified glassy carbon electrode for the detection of dopamine. Wu et al. [4] have shown the selective determination of dopamine at glassy carbon electrodes modified with an over-oxidized poly (*N*-acetylaniline). Ogorevc and Mo [5] have recently proposed the use of carbon fiber microelectrodes coated with over-oxidized poly-(1,2-phenylenediamine) to obtain a highly sensitive and selective dopamine quantification. We have also reported on the use of a glassy carbon electrode modified with a melanin-type polymer electrogenerated from L-dopa for the highly selective dopamine quantification [6]. Chen and Link have shown the electrocatalytic activity of polymerized luminal film-modified electrodes that allows the selective measurement of dopamine and ascorbic acid [7]. Ohsaka et al. have reported on the use of a glassy carbon electrode modified with an electropolymerized film of *N,N*-dimethylaniline for the sensitive and selective quantification of dopamine [8].

Several polymeric membranes have been also used to improve the selectivity of glucose determinations using amperometric biosensors based on the detection of hydrogen peroxide enzymatically generated [1, 2, 9–11]. In this case, the polymer works as a barrier to reject AA and uric acid (UA), compounds usually present in blood. It is important to mention that the electropolymerization was used not only to reject usual interferents but also to immobilize GOx at the electrode surfaces [1, 2, 10].

It is widely known that melanic polymers can be obtained from catechols in the presence of polyphenol oxidase, that catalyzes their aerobic oxidation to the corresponding *o*-quinones (Scheme 1) [12]. It is also known that catechols can be easily electrooxidized to the corresponding quinones and

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then spontaneously converted into melanic polymers [13]. The electrooxidation of catechols at all pH values starts with the generation of the *o*-quinone. Above pH 4.0 these quinones rearrange by cyclization of the side chain to be transformed into the leuco-derivative, which is more easily oxidized than the corresponding quinone that can be oxidized to dopachrome and subsequently transformed into melanic polymers. We have reported recently on the generation of a melanin-type polymer from the electrooxidation of L-dopa on glassy carbon electrodes [6] as well as on other carbonaceous materials like carbon fiber, glassy carbon paste, graphite paste, graphite and screen printed electrodes [14]. A potential of 1.0 V is enough to produce the polymerization of a 3.0×10^{-3} M L-dopa solution and the resulting polymeric layer demonstrated to be highly successful in the rejection of anionic compounds.

In this paper we report on the properties of polymers electrogenerated from different catechols ranging from the unsubstituted catechol to different catecholamines like dopamine, epinephrine and norepinephrine in addition to L-dopa, either in batch or in flow systems. The analytical applications of these polymeric layers were evaluated in connection with the detection of dopamine and glucose.

2. Experimental

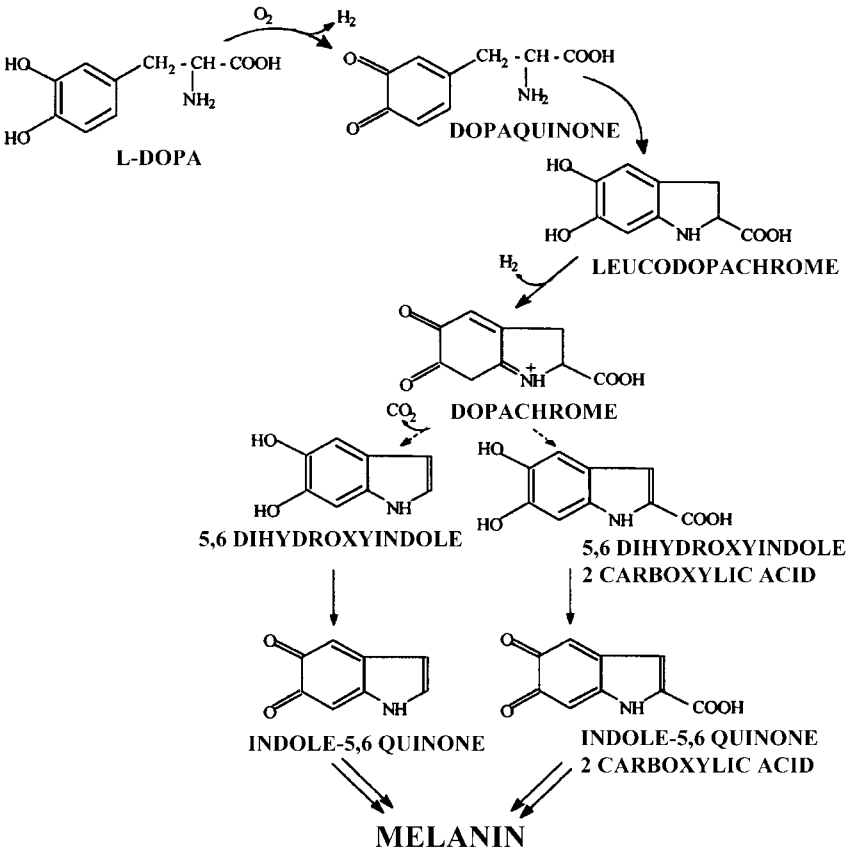
2.1. Reagents

3,4-Dihydroxyphenyl acetic acid (dopac), 3-hydroxytyramine (dopamine), L-β-3,4-dihydroxyphenylalanine (L-dopa), pyrocatechol, arterenol ([±]-norepinephrine) hydrochloride and (±) epinephrine (4-[1-hydroxy-2-(methylamino)-ethyl] 1,2 benzenediol) hydrochloride, glucose oxidase ((GOx) (Type X-S, *Aspergillus niger*, (EC 1.1.3.4), 210000 Units per gram of solid, Catalog number G-7141), ascorbic acid (AA), uric acid (UA) and acetaminophen were from SIGMA. Other chemicals were reagent grade and used without further purification.

Ultrapure water ($\rho = 18$ MΩ) from a Millipore-MilliQ system was used for preparing all the solutions. A 0.050 M phosphate buffer solution pH 7.40 was used as supporting electrolyte for cyclic voltammetry and for amperometric experiments in batch while the same buffer containing also 1.0×10^{-3} M ascorbic acid was used in the flow injection experiments.

2.2. Apparatus

The voltammetric and amperometric experiments were performed with an Epsilon potentiostat (BAS). The electro-



Scheme 1. Enzymatic generation of melanin from L-dopa.

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des were inserted into the cell (BAS, Model VC-2) through its Teflon cover. A Ag/AgCl, 3 M NaCl (BAS, Model RE-5B) and a platinum wire were used as reference and counter electrodes, respectively. All the potentials are referred to that reference. A magnetic stirrer and a stirring bar were used for the convective transport when necessary.

Flow injections (FIA) experiments were performed with an Amperometric Detector LC 4-C from BAS connected to a Pelkin-Elmer 56 recorder. The electrodes were inserted into a wall-jet cell (EA-1096 Methrom) containing a gold wire as counter electrode and a Ag/AgCl, 3 M NaCl (Methrom) as reference electrode. The working electrode was a glassy carbon (BAS) (GCE). A peristaltic pump (ISMATEC, ISM 834) was used to provide the flow during the experiments. The valve was a Rheodyne type 50, four-way loop pressure valve (Altech) (100 μL sample loop). The connections were made with an interconnecting PTFE tubing (0.8 mm i.d.).

The scanning electronical microscopy (SEM) studies were performed with a PHILIPS XL30 scanning electronical microscope coupled to an EDAX DX4i analyzer.

2.3. Electrode Preparation and Procedure

The glassy carbon electrode was cleaned by hand polishing using first emery paper (600) for 2 min, then emery paper (BAS) for 2 additional min, followed by fine grades of alumina slurries of 1.0, 0.3 and 0.05 μm (Buehler) for 2 minutes in each step with exhaustive rinsing after that. Once polished, the electrode was checked by cyclic voltammetry in phosphate buffer solution between −0.40 and 0.80 V.

Polymer-modified electrodes were obtained by applying a constant potential of 1.0 V for 60 min using a stirred air saturated 0.050 M phosphate buffer solution pH 7.40 containing 3.0×10^{-3} M of catechol or a given catecholamine (either L-dopa, dopamine, norepinephrine or epinephrine).

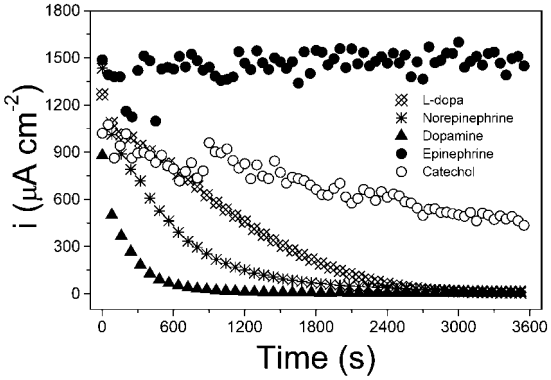

Fig. 1. Chronoamperometric recordings for the electropolymerization of different catechols at glassy carbon electrodes. Electropolymerization conditions, 60 min at 1.0 V in supporting electrolyte containing 3.0×10^{-3} M of the corresponding catechol. Supporting electrolyte: 0.050 M phosphate buffer solution pH 7.40.

Once the polymer was obtained, the electrode was washed with water and cycled in supporting electrolyte between −0.40 V and 0.80 V at 0.100 V/s (5 cycles). For FIA experiments a constant potential of 0.700 V was applied and once the current decays to a constant value, the measurements start.

Enzymatic metallized electrodes (CPE-Pt-GOx) were prepared in the following way: the desired amount of enzyme (usually 15.0% w/w) was mixed with mineral oil (Aldrich) in an agata mortar for 3 minutes followed by the incorporation of graphite powder containing 5.0 or 10.0% w/w platinum on Vulcan XC-72 (E-TEK, Inc) and mixing for 15 additional minutes. Carbon paste electrodes (CPE) containing GOx (CPE-GOx) were prepared in a similar way by using graphite powder (Fisher # 38) instead of the metallized carbon. A portion of the resulting paste was packed firmly into the cavity (3.0 mm diameter) of a Teflon tube. The electric contact was established through a stainless steel screw. A new surface was obtained by smoothing the electrode onto a weighing paper. The melanin-type polymer was obtained by applying 1.00 V for 120 min using a stirred air saturated 0.050 M phosphate buffer solution pH 7.40 containing 3.0×10^{-3} M dopamine. Once the polymer was obtained on the surface of the enzymatic electrode, it was washed with water and cycled in supporting electrolyte between −0.40 V and 0.80 V at 0.100 V/s (1 cycle).

3. Results and Discussion

3.1. Glassy Carbon Electrode Modified with Polymers Electrogenerated from Different Catechols. Characterization and Analytical Applications for Dopamine Quantification

Figure 1 shows $I-t$ profiles for the electrolysis of different catechols at 1.00 V on glassy carbon electrodes. During the electrolysis of dopamine, L-dopa and norepinephrine an additional process besides that associated with the catechol/catechol-quinone couple was observed at short times due to the formation of the catechol-chrome compound. After that, since the polymerization takes place, the currents largely decreased. The fastest decrease was observed during the polymerization of dopamine while the slowest during the polymerization of L-dopa. In the case of epinephrine electrolysis, the signal was very noisy and it remained almost without change during the entire period, indicating that the efficiency of the polymerization under these conditions was very poor. When catechol was electrooxidized, the signal was also noisy but despite that the current decreased with time, after one hour polymerization it remained ca. 50% of the initial value. These results suggest that the nature of the polymer is highly dependent on the nature of the catechol used as monomer, as discussed below.

In order to evaluate the permselective properties of the electrogenerated polymers, ascorbic acid, dopac, uric acid, potassium ferrycyanide, dopamine, epinephrine and cate-

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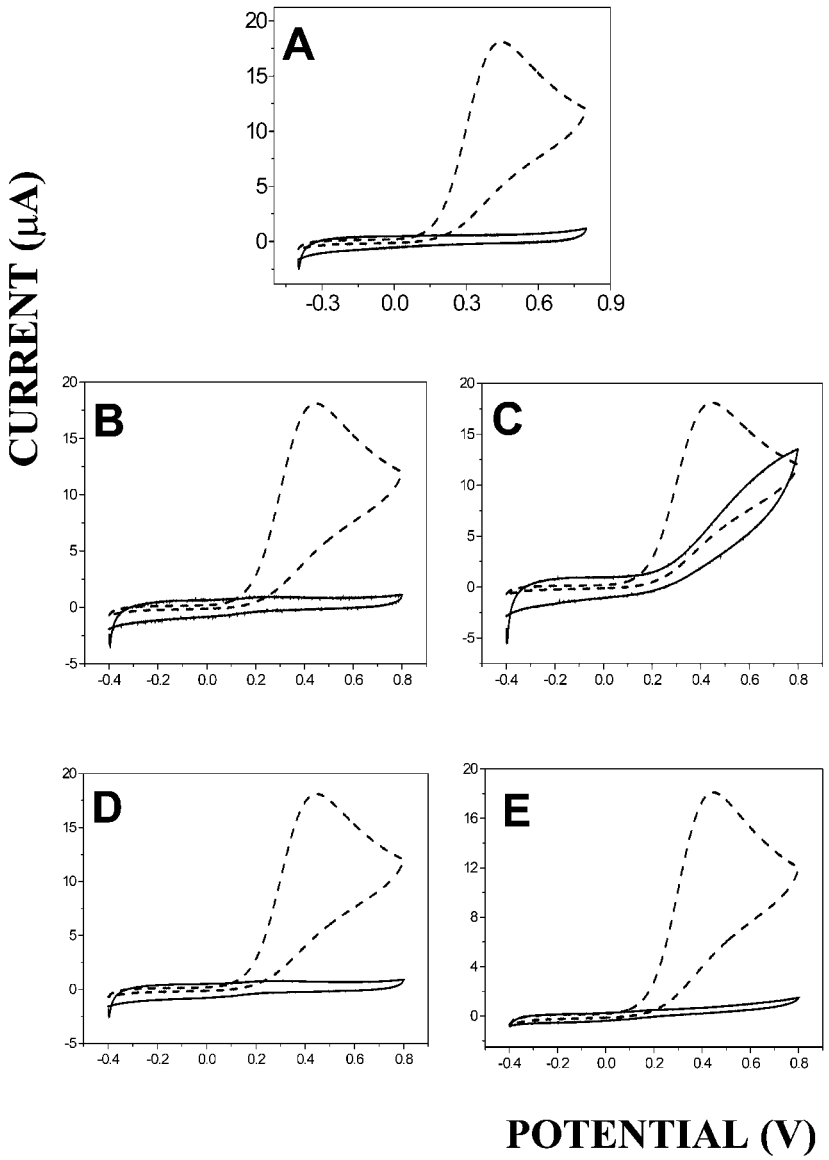

Fig. 2. Cyclic voltammograms for 1.0×10^{-3} M ascorbic acid at bare (dotted line) and polymer-modified (solid line) glassy carbon electrodes. Polymers electrogenerated from L-dopa (A), norepinephrine (B), epinephrine (C), dopamine (D) and catechol (E). Scan rate: 100 mV/s. Electropolymerization conditions as in Figure 1.

chol were used as indicators. Figure 2 shows the voltamperometric response of 1.0×10^{-3} M ascorbic acid (pK 4.10) at bare glassy carbon (dotted lines) and at glassy carbon modified with polymers electrogenerated from solutions containing 3.0×10^{-3} M L-dopa (A), norepinephrine (B), epinephrine (C), dopamine (D) and catechol (E) (solid lines). The peak potential for the oxidation of AA at bare glassy carbon was 0.43 V while the oxidation current at this potential was 18.3 μA. As can be seen, an important decrease in the oxidation currents at the peak potential, that depends on the nature of the monomer used to perform the electropolymerization, was observed at the different polymer-modified glassy carbon electrodes. Decreases of 99.9, 99.9, 73.6, 100 and 95.2% were obtained at glassy carbon electrodes modified with the polymers generated from L-dopa, norepinephrine, epinephrine, dopamine and catechol, respectively.

A large decrease in the oxidation currents at the polymer modified electrodes was obtained in similar experiments using dopac (pK 4.22) as a marker. The oxidation current (17.9 μA) at the peak potential (0.54 V) decreased 99.8, 99.9, 73.1, 99.3 and 96.9% at glassy carbon modified with the polymers generated from L-dopa, norpinephrine, epinephrine, dopamine and catechol, respectively. The response of uric acid (pK 5.1) and potassium ferrycyanide at the glassy carbon modified electrodes was also evaluated and it was similar to that obtained for ascorbic acid and dopac (not shown).

The electrochemical behavior of neutral compounds such as catechol and cationic ones like dopamine (pK 8.2) and

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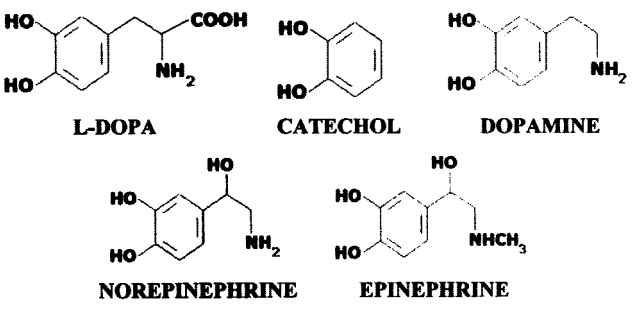
epinephrine (pK 9.6) at the polymer-modified electrodes was also studied (not shown). In general, the oxidation currents were slightly higher at the modified electrodes, with exception to the electrode modified with a polymer electrogenerated from dopamine, where a large decrease in the oxidation currents was observed for all the compounds, indicating that the polymeric layer represents an important diffusional barrier.

Regarding the electrochemical behavior of dopamine (1.0×10^{-3} M) at glassy carbon electrodes modified with the polymer electrogenerated from catechol, no significant changes were observed compared to that at bare glassy carbon electrode. On the contrary, the oxidation of dopamine at glassy carbon electrode modified with the melanic polymer generated from L-dopa occured at potentials 110 mV less positive than at bare glassy carbon electrode and the oxidation current was around 72% higher. The oxidation current at glassy carbon modified with the melanic polymer generated from dopamine was 97.4% smaller than that obtained at the bare glassy carbon, meaning that, even when dopamine is positively charged, the barrier effects of the polymer are very important. However, the response was more reversible than at glassy carbon electrode modified with the other polymers, with i_{pa}/i_{pc} of 1.53 and ΔE_p of 0.180 V, values similar to those obtained at glassy carbon electrode modified with the polymer generated from L-dopa. An intermediate behavior was observed at GCEs modified with the polymers obtained from norepinephrine and epinephrine (not shown).

Therefore, all the polymers generated from the different catechols used in this work reject negative compounds and their permselective properties depend on the catechol used for the electropolymerization. The best rejection was obtained with the polymer electrogenerated from dopamine, while the worst, with the one generated from epinephrine. By evaluating the structure of the catechol used to perform the electropolymerization (Scheme 2) and the permselective properties of the resulting polymer, some correlation can be done. Since catechol, at variance with catecholamines, does not present a side chain, the cyclization of the quinone to give the leucocompound (Scheme 1) does not occur and, in consequence, the melanic polymer can not be formed. The resulting polymer possesses good barrier properties towards negative compounds although they are not as good as in the case of the melanic polymers (generated from catecholamines). Regarding the electropolymerization of catecholamines, since all of them have a side chain, the cyclization of the corresponding quinones takes place and the melanin-type polymers can be formed. L-dopa, dopamine and norepinephrine present a primary amine group in the side chain, which is involved in the further conversion of the quinones into indols and melanic polymers. At variance with these catecholamines, epinephrine presents the amine group substituted with a methyl residue, making the subsequent polymerization more difficult. Since the most effective rejection of ascorbic acid was obtained with polymers generated from L-dopa, norepinephrine and dopamine and the poorest occurred with that generated from epinephrine (Fig. 2), it is clear that the polymers with the best permselective properties are those obtained from catecholamines containing a primary amine group in the side chain like in the case of L-dopa, dopamine and norepinephrine and that the presence of an additional substituent in this amine group makes the resulting polymer less effective to reject anions.

The behavior of the glassy carbon electrodes modified with the different polymers in flow systems was also evaluated using 1.0×10^{-6} M dopamine as well as ascorbic acid and dopac at their maximum physiological levels in nervous centers (physiological values in nervous centers: from 1.0×10^{-4} M to 5.0×10^{-4} M for ascorbic acid and from 1.0×10^{-6} M to 2.0×10^{-5} M, for dopac [15]) as indicators of the permselective properties of the polymers. The carrier was a 0.050 M phosphate buffer solution pH 7.40 containing 1.0×10^{-3} M ascorbic acid. The goal of the incorporation of this compound in the carrier solution was to amplify the dopamine oxidation signal due to the chemical reaction between ascorbic acid and the dopaminequinone electrochemically generated [6]. Consequently, ascorbic acid is used both, to improve the sensitivity of dopamine quantification and to evaluate the barrier properties of the electrogenerated polymers.

Figure 3A shows the amperometric response of a glassy carbon electrode modified with a melanic polymer generated from L-dopa after successive injections of dopamine (1.0×10^{-6} M), ascorbic acid (5.0×10^{-4} M) and dopac (5.0×10^{-5} M) at a potential of 0.700 V and a flow rate of 2.2 mL/min. The signal for dopamine oxidation clearly increases after the successive injections to remain almost constant between 40 and 125 min at values which are double the first one. After that, the signal starts to decrease almost reaching the initial value at 250 min, probably due to the accumulation of some oxidation residue that passivates the electrode (not shown). Another important aspect to be considered was the permselectivity of the polymer towards ascorbic acid and dopac. The interference of these compounds slightly decreases with the successive additions of dopamine and remain constant after 60 min. After 250 min, as in the case of dopamine, the oxidation current for ascorbic acid and dopac also decreases, confirming that some passivation of the electrode is occurring (not shown).



Scheme 2. Estructure of the different catechols used as monomers for the electropolymerization.

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As depicted in Figure 3B, in the case of the glassy carbon electrode modified with the polymer electrogenerated from norepinephrine the behavior was similar to that obtained for the melanic polymer generated from L-dopa. Dopamine oxidation signal increases after the successive additions of 1.0×10^{-6} M dopamine and remains almost constant between 80 and 175 min at a value around 3 times higher than the first one. At variance with the behavior of glassy carbon electrodes modified with the melanic polymer generated from L-dopa, the response for dopamine remains relatively constant even after 225 min, indicating that the passivation of the electrode is less pronounced. Regarding the interferents behavior, the ascorbic acid oxidation signal slightly increases up to 150 min, to remain almost constant after that. For dopac the signal slightly increases with time. Despite the barrier properties towards negative compounds are clear, in all cases the interference percentages were higher than those observed when using a glassy carbon electrode modified with the polymer generated from L-dopa.

The flow injection response of a glassy carbon electrode modified with a polymer electrogenerated from dopamine

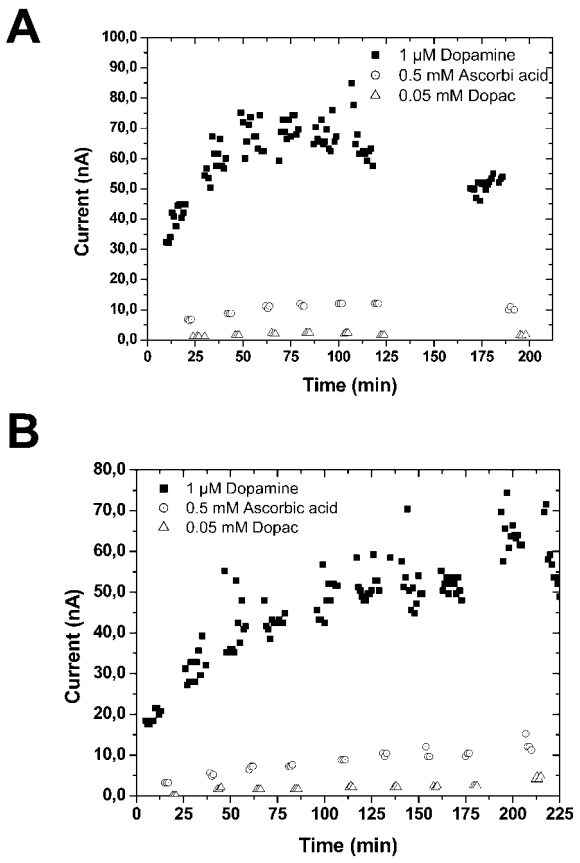

Fig. 3. Oxidation current for 1.0×10^{-6} M dopamine, 5.0×10^{-4} M ascorbic acid and 5.0×10^{-5} M dopac at glassy carbon electrodes modified with melanin-type polymers electrogenerated from L- dopa (A) and norepinephrine (B). Electropolymerization conditions: as in Figure 1. Flow rate: 2.2 mL/min. Carrier: 0.050 M phosphate buffer pH $7.40 + 1.0 \times 10^{-3}$ M ascorbic acid. Working potential: 0.700 V.

was also evaluated (not shown). At variance with the response obtained at glassy carbon electrodes modified with polymers generated from L-dopa and norepinephrine, the oxidation signal was less sensitive and it continuously increased after the successive additions of 1.0×10^{-5} M dopamine. Since the oxidation signal of ascorbic acid and dopac remained almost constant, the selectivity largely improves after the successive additions of dopamine. For instance, after 200 min use the interference percentages were 6.1% and 5.2% for ascorbic acid and dopac, respectively. However, despite this excellent selectivity, the sensor is not useful for practical applications in the quantification of dopamine, since it is not possible to get a stable oxidation signal in a reasonable time.

The behavior of a glassy carbon electrode modified with a polymer generated from catechol in flow systems was also studied (not shown). In view of the fact that the barrier properties of this polymer are not as good as those of the melanic polymers, it was not possible to work with a carrier containing 1.0×10^{-3} M ascorbic acid due to the important increase in the background currents. After successive injections of 1.0×10^{-6} M dopamine its oxidation signal decreases. For instance, after 150 min use the signal for 1.0×10^{-6} M dopamine was around 60% smaller than the first one, while the interferences for ascorbic acid and dopac were 100% and 50% higher, respectively. These results, in agreement with those shown in Figure 2, indicate that the polymer is not stable enough to be used in flow systems and that some passivation occurs on the surface of the electrode after successive additions of dopamine. Similar behavior was observed in the case of the polymer obtained from epinephrine.

The response of a bare glassy carbon electrode after successive additions of 1.0×10^{-6} M dopamine was also evaluated (not shown). The oxidation signal for the first addition of dopamine was 240 nA, decreasing to 40 nA in the fifth injection. This important decrease in the oxidation signal is due to some fouling effect on the surface of the electrode. As expected, according to Figure 1, a huge interference is obtained for dopac and ascorbic acid.

Figure 4 shows SEM photographs of glassy carbon surfaces before (A) and after the electropolymerization of L-dopa (B) and dopamine (C). It is possible to see that once the polymerization of L-dopa takes place, the surface of the glassy carbon electrode is covered by a homogeneous and spongeous film (Fig. 4B). Small black deposits are also observed on the whole surface due to the formation of the melanic polymer. Similar deposits were observed when depositing commercial melanin (not shown). In the case of the surface covered by the melanic polymer generated from dopamine (Fig. 4C), the morphology was different. The film seems to be thinner than that obtained from L-dopa and the deposit is more evident following the polishing lines.

In summary, the behavior of the polymers generated by electrooxidation of different catechols at glassy carbon can be summarized as follows:

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– The polymers generated from catechol, L-dopa, dopamine, epinephrine and norepinephrine present permselective properties towards negatively charged compounds that depends on the nature of the monomer.
– The polymers generated from L-dopa, dopamine and norepinephrine (for times shorter than 200 min) prevent the fouling of the electrode.
– The polymers generated from epinephrine and catechol are not useful for continuous monitoring of dopamine due to the fouling of the modified electrode.
– The selectivity of the glassy carbon electrode modified with the polymer generated from dopamine (for dopamine quantification) is excellent, however, the sensitivity of this electrode is very poor and the oxidation signal is not stable enough to ensure reproducible dopamine measurements.
– The glassy carbon electrode modified with the polymer generated from norepinephrine allows a highly sensitive dopamine quantification, although the response is less selective than in the case of glassy carbon electrode modified with polymers generated from L-dopa or dopamine.
– The polymer that allows one to obtain a modified glassy carbon electrode with the best compromise between stability, sensitivity and selectivity in the quantification of dopamine, either in batch or in flow systems, is the one generated from the electropolymerization of L-dopa.

The flow rate was shown to be an important variable in the analytical performance of sensors when using flowing streams. The effect of the flow rate on the oxidation signal of dopamine at 0.700 V using a glassy carbon electrode modified with a melanic polymer generated from L-dopa was evaluated over the 0.6 to 5.7 mL/min range (not shown). At lower flow rates, as expected, the dispersion was very important and, consequently the peaks were very broad and the signals less sensitive. At flow rates higher than 2.2 mL/min there was a decrease in the response because the interaction time between dopamine and the electrode was not long enough to allow the amplification of the dopamine oxidation. The best compromise was obtained with 2.2 mL/min.

Figure 5 depicts the amperometric response of 1.0×10^{-6} M dopamine, 5.0×10^{-4} M ascorbic acid and 5.0×10^{-5} M dopac at a glassy carbon electrode modified with a melanic polymer generated from L-dopa after conditioning at a flow rate of 2.2 mL/min. The interference of 5.0×10^{-4} M ascorbic acid was 9.0% while that for 5.0×10^{-5} M dopac was 2.0%. The response of the modified electrode towards dopamine was highly reproducible with an average current of 82.3 nA and a R.S.D. of 2.6%.

Figure 6 depicts the FIA i-t recordings (FIAgrams) obtained at a glassy carbon electrode modified with a melanic polymer generated from L-dopa for different concentrations of dopamine from 5.0×10^{-9} M to 5.0×10^{-7} M at a potential of 0.700 V and a flow rate of 2.2 mL/min. As it can be seen, well defined peaks were obtained even for namolar levels of dopamine. The calibration plot

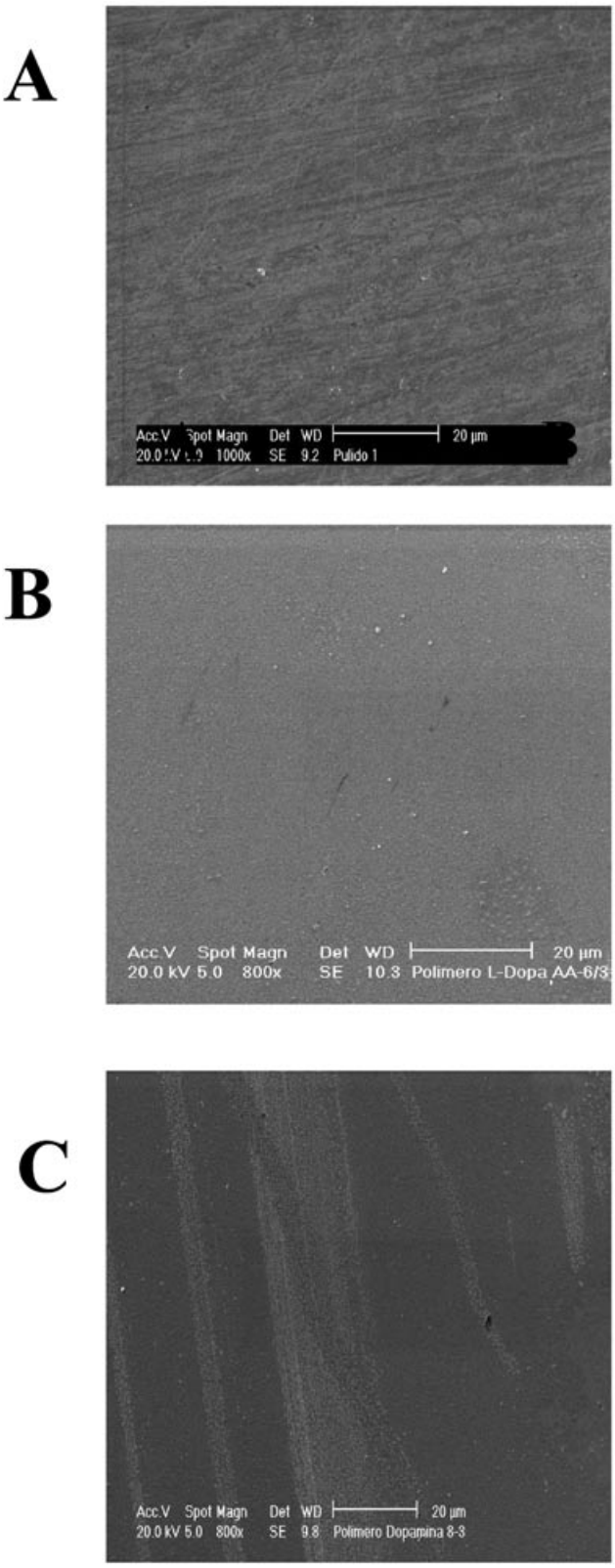

Fig. 4. SEM photographs for polished glassy carbon electrode (A) (1000 ×), melanin-type polymer modified glassy carbon electrode generated using L-dopa (B) (800 ×) or dopamine (C) (800 ×) as monomers.

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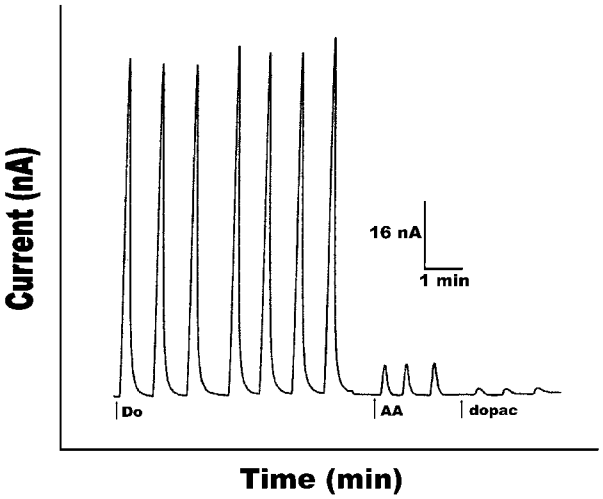

Fig. 5. Fiagrams obtained for 1.0×10^{-6} M dopamine (Do), 5.0×10^{-4} M ascorbic acid (AA) and 5.0×10^{-5} M dopac at glassy carbon electrodes modified with a melanin-type polymer electrogenerated from L- dopa. Electropolymerization conditions: as in Figure 1. Flow rate: 2.2 mL/min. Carrier: 0.050 M phosphate buffer pH $7.40 + 1.0 \times 10^{-3}$ M ascorbic acid. Working potential: 0.700 V.

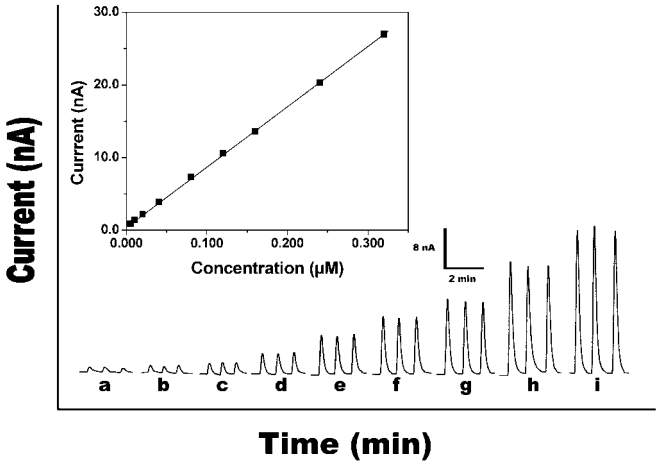

Fig. 6. Fiagrams obtained for different dopamine concentrations: 5.0×10^{-9} (a), 1.0×10^{-8} (b), 2.0×10^{-8} (c), 4.0×10^{-8} (d), 8.0×10^{-8} (e), 1.2×10^{-7} (f), 1.6×10^{-7} (g), 2.4×10^{-7} (h) and 3.2×10^{-6} (i). The corresponding calibration plot is also shown as an inset. Other conditions as in Figure 5.

shows a linear relationship between 5.0×10^{-9} and 3.50×10^{-7} M with a sensitivity of $(8.24 \pm 0.03) \times 10^{7}$ nAM^{-1}, a correlation coefficient of 0.9994 ($n = 9$) and a detection limit of 5.0×10^{-9} M.

3.2. Use of the Melanin-Type Polymer to Develop Glucose Biosensors

As already discussed in the previous section, the polymer electrogenerated from dopamine allows us to obtain an excellent discrimination against negatively charged compounds. Therefore, based on the excellent permselective properties of this polymer (Figure 1), it was used as a barrier to reject interferents in the preparation of highly selective glucose biosensors.

In this sense, it is interesting to consider the effect of the melanic polymers on the oxidation of hydrogen peroxide, compound involved in the enzymatic oxidation of glucose (not shown). The general behavior is that the oxidation of hydrogen peroxide at the melanin-type polymers modified glassy carbon electrodes starts at lower potentials and the oxidation currents are larger than that at bare glassy carbon electrodes. Even at the glassy carbon electrode modified with the polymer electrogenerated from dopamine, which represent an important diffusional barrier, the response for hydrogen peroxide was more sensitive than that at the bare glassy carbon electrode. This behavior suggests that the polymers electrogenerated from catechol and catecholamines present some catalytic effect on the oxidation of hydrogen peroxide that also depends on the nature of the polymer. However, although these polymeric layers present a catalytic effect towards hydrogen peroxide oxidation, the signal for the oxidation of the enzymatically generated hydrogen peroxide at carbon paste containing GOx and covered by the melanic polymer generated from dopamine, was not high enough to allow the selective determination of glucose.

It is widely known that platinum catalyzes the oxidation and reduction of hydrogen peroxide [16]. However, it presents an inconvenience in catalyzing the oxidation of the usual interferents such as ascorbic acid and uric acid. Here we are reporting on a new biosensor based on a carbon paste electrode containing platinum microparticles covered by a melanic polymer generated from dopamine in order to improve the sensitivity and selectivity of the glucose biosensing.

Figure 7A shows the amperometric response at 0.700 V of a biosensor prepared by dispersion of 15.0% w/w GOx in a composite made of 5.0% w/w Pt-Vulcan carbon and mineral oil, and covered by an electrogenerated melanic polymer after one addition of 5.0×10^{-3} M glucose and the usual interferentes (ascorbic acid, uric acid and acetaminophen). The biosensor responds rapidly to the glucose susbtrate. In fact, a steady-state current was attained after 10 seconds of the addition of glucose. The following addition of ascorbic acid in a concentration more than five times higher than the maximum physiological level in blood (physiological range, from 2.0×10^{-5} M to 7.6×10^{-5} M [17]) produced a direct electrooxidation signal which was just 2.0% of the oxidation signal obtained after adding 5.0×10^{-3} M glucose (2.0% interference). The injection of 5.0×10^{-4} M uric acid (8.4 mg%), a concentration even higher than the maximum physiological level (physiological range between 3.0 and 6.0 mg% [17]), produced only 3.0% interference. Finally, the addition of 1.0×10^{-4} M acetaminophen interfered only in a 2.5%. Therefore, the advantages in the selectivity of the glucose biosensing of this new biorecognition layer are clearly demonstrated.

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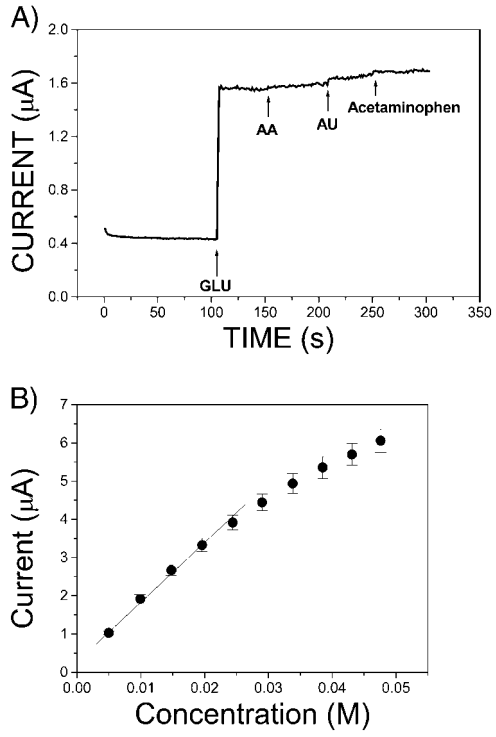

Fig. 7. A) Current-time profiles at CPE-Pt (5.0% w/w)-GOx-(15.0% w/w)-mel for one addition of 5.0×10^{-3} M glucose (GLU) followed by additions of 5.0×10^{-4} M ascorbic acid (AA), 5.0×10^{-4} M uric acid (UA) and 1.0×10^{-4} M acetaminophen (acet). Operating potential: 0.700 V. Electropolymerization conditions: 60 min at 1.00 V using a stirred air saturated 0.050 M phosphate buffer solution pH 7.40 containing 3.0×10^{-3} M dopamine. B) Average calibration plot obtained from eight amperometric recordings performed with the same electrode.

Figure 7B depicts a calibration plot obtained as the result of eight calibrations performed with the same surface for glucose levels between 5.0×10^{-3} M and 5.0×10^{-2} M. The analytical characteristics are the following: linear range up to 2.5×10^{-2} M (4.50 g/L), detection limit of 143 μM, sensitivity of (156 ± 6) μA M^{-1} and $r = 0.998$. Similar experiments, but using 6 different electrodes gave a slope of (187 ± 8) μA M^{-1}, $r = 0.997$. As the errors in sensitivities indicate, the response of the bioelectrodes is highly reproducible, being, in all cases 2.5×10^{-2} M glucose the upper limit of the dynamic linear range.

4. Conclusions

This study has clearly demonstrated that the permselective properties of the polymers electrogenerated from different catechols largely depend on the structure of the catechol, being necessary the presence of a side chain containing a non substituted amine group (like in the case of L-dopa, dopamine and norepinephrine). The usefulness of the glassy carbon electrodes modified with these polymers in flow systems was also demonstrated. The stability of the bio-recognition layer is a function of the nature of the polymer, the one obtained from the electropolymerization of norepinephrine and L-dopa being the best. The most sensitive, selective, fast and stable response to dopamine is obtained using a glassy carbon electrode modified with the melanic polymer electrogenerated from L-dopa.

The excellent permselective properties of the melanin-type polymer generated from dopamine at the surface of CPE containing GOx and Pt, has allowed us to obtain a dramatic improvement in the selectivity of the glucose biosensor. The combination of the advantages of this polymeric layer with the excellent catalytic properties of Pt towards the hydrogen peroxide electrooxidation, results in a highly selective and sensitive glucose biosensor. This biosensor has allowed us to get a response even more selective than the one obtained with a CPE-Pt-GOx covered by a melanin-type polymer generated from L-dopa [18]. Another important advantage is that, due to the better permselective characteristics of the polymer generated from dopamine, the preparation of the enzymatic electrode is faster than that using the polymer generated from L-dopa.

Therefore, the analytical application of the melanic polymers has been demonstrated. Although these properties have been shown in connection to dopamine and glucose quantification, they could be also used for the determination of other bioanalytes.

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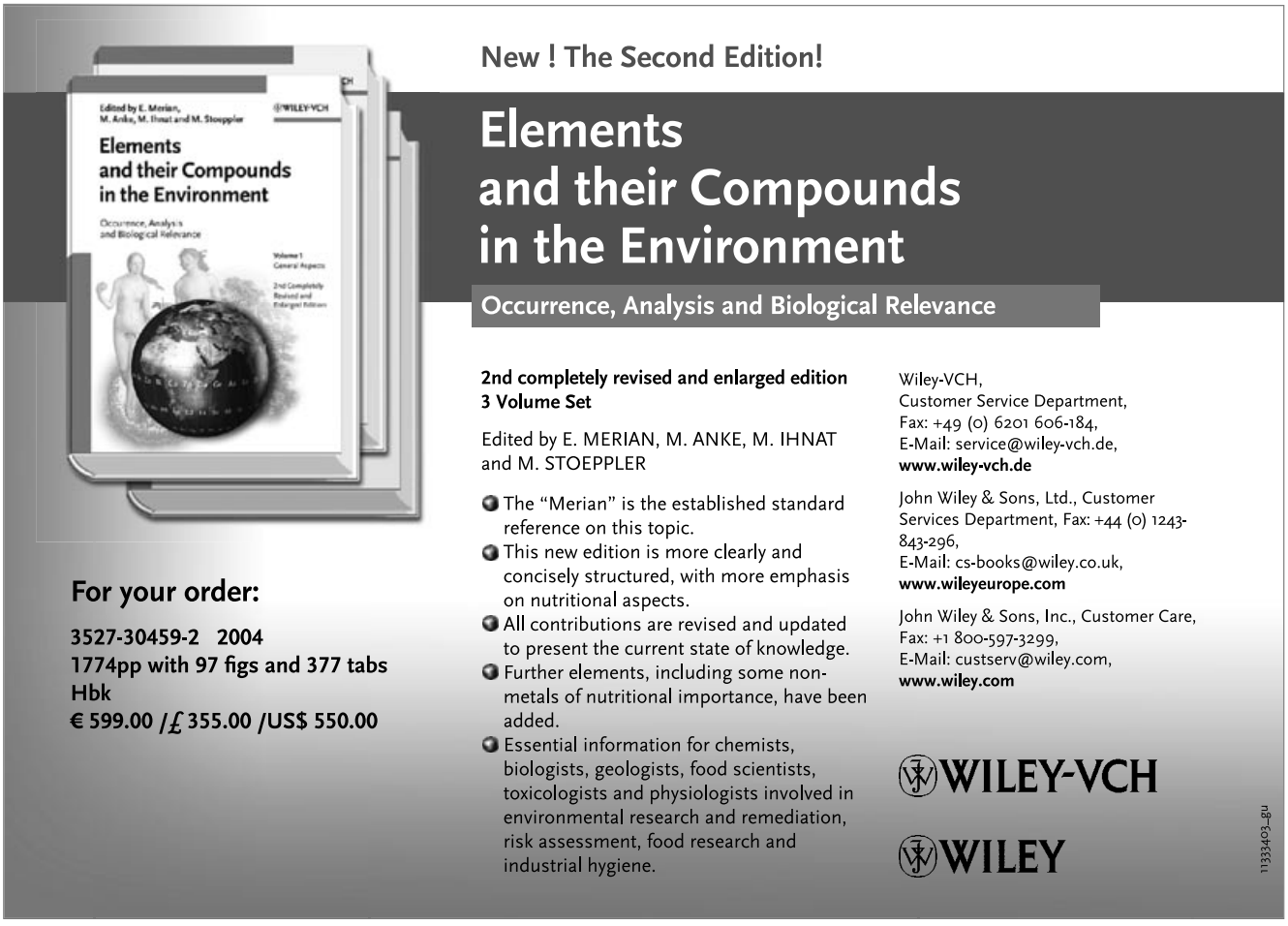

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