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Flow injection-pulse amperometric detection of ephedrine at a cobalt phthalocyanine modified carbon paste electrode

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Direct detection of ephedrines and other underivatized amino compounds (amines, alicyclic amines, alkanolamines, and amino acids) can be carried out *via* electrocatalytic oxidation at a carbon paste electrode (CPE) modified with cobalt phthalocyanine (CoPC) in alkaline solution (0.10 mol L^{-1} NaOH). Most of the amino compounds tested could be determined using the CoPC/CPE in an amperometric flow detector. The analytical signal of ephedrine was stabilised by alternating the potential between an anodic detection potential of +0.30 V (+0.45 V for other amino compounds) applied for 220 ms and a cathodic reactivation potential of −0.30 V applied for 100 ms (potentials *versus* SCE). The linear response range for ephedrine was within 1–100 μmol L^{-1} and the detection limit was 0.8 μmol L^{-1} with a 100 μL sample loop and a typical sampling rate of 60 h^{-1}. The signal (oxidation peak current) reproducibility was 2–3%. The method was applied to the determination of ephedrine in pharmaceutical formulations with results comparable to those obtained with a standard spectrophotometric method.

Introduction

After their introduction by Baldwin *et al.*,[1] carbon paste electrodes (CPEs) modified with cobalt phthalocyanine (CoPC) have been used for the amperometric detection of compounds that are otherwise non-electroactive or oxidisable only at extreme potentials. Applications of CoPC-modified CPEs (CoPC/CPEs) have been previously summarized.[2] To the best of our knowledge, the electrochemical behaviour of amino compounds at CoPC-modified electrodes has not been examined.

Ephedrine is a naturally occurring sympathomimetic drug that stimulates both α- and β-adrenergic receptors. It causes a rise of systolic and diastolic pressure, bronchodilation and mild stimulation of the central nervous system (CNS). Ephedrine has been used at therapeutic doses of 15–60 mg in the treatment of asthma, allergic states, catalepsy and myasthenia gravis, to raise the arterial pressure, as a nasal decongestive, as an antidote for poisoning by CNS depressants and in spinal anaesthesia.[3] Ephedrine has been characterised as a prohibited compound by the International Olympic Committee.[4] As ephedrine is not only an ingredient of common anti-cold preparations but also of various nutritional supplements, athletes tested positive for ephedrine often claim to have received it while using products without indication. Therefore, the determination of ephedrine in biological fluids and pharmaceutical preparations *via* a simple and reliable method is of great interest. An indirect determination of ephedrine in urine by differential-pulse polarography has been reported by Hernandez *et al.*[5] An extensive list of other techniques for the determination of ephedrine can be found therein.

In this work, the electrochemical oxidation of ephedrine and of other organic amino compounds at a CoPC/CPE was examined and the electrode was used as a detector in flow injection amperometric determinations. The method was successfully applied to the determination of ephedrine in pharmaceutical preparations.

Experimental

Reagents

Ephedrine [(1*R*,2*S*)-(−)-α-(1-methylaminoethyl)benzyl alcohol] and norephedrine [α-(1-aminoethyl)benzyl alcohol], as the hydrochloride salts, and *N*-methylephedrine, as the free base, were obtained from Aldrich (Steinheim, Germany) (purity ⩾99%). All other amino compounds were obtained from Merck (Darmstadt, Germany) or Fluka (Buchs, Switzerland) and were of the purest reagent quality. All dilute solutions were prepared fresh when needed by diluting stock solutions (0.100 mol L^{-1}). De-ionised, distilled water was used throughout.

NaOH solutions in the concentration range 0.001–1 mol L^{-1} and Britton–Robinson (B–R) buffer solutions were used throughout as supporting electrolytes.

Electrodes

Carbon paste for unmodified electrodes, 63% m/m in graphite, was prepared by thoroughly mixing in a mortar dry graphite powder (Fluka) and Nujol (Merck, IR grade). Carbon paste for modified electrodes, 62% m/m in graphite and 2% m/m in CoPC, was prepared in a similar way except that the appropriate amount of CoPC was first mixed with the graphite. Polyethylene syringes (1 mL), the tips of which had been cut off with a scalpel, were packed with 0.5–1 g of carbon paste. Electrical contact was established *via* a copper wire passing through the piston.

Apparatus

All measurements (cyclic voltammetry, pulse amperometry) were performed using the potentiostat of the Tacussel (Lyon, France) PRG-5 polarograph. The flow injection system consisted of a Tecator (Höganas, Sweden) FIAstar 5020 analyzer

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coupled to a laboratory-built Plexiglas 'wall-jet' type flow amperometric detector, previously described.[2] All potential measurements reported hereafter are referred to the potential of the saturated calomel reference electrode (SCE). All flow injection measurements were obtained with a flow rate of 1.00 mL min^{-1}, a 100 μL sample loop and a measurement rate of 60 h^{-1}.

A 486 DX-40 PC was interfaced to the polarograph and the analyser and control programs written in Delphi for Windows (CV experiments) and in Turbo-Pascal (FIA measurements), both products of Borland-Inprise (Scotts Valley, CA, USA), were used. The FIA control program provided control of the injection valve and sample/wash timing, free selection of the applied potential waveform to the potentiostat, sampling of current indications from the polarograph output (*via* a 12-bit analogue-to-digital converter), and displaying of the current peaks with automatic evaluation of the peak height.

Results and discussion

Cyclic voltammetry

In strongly alkaline solutions (0.1–1 M NaOH) most of the amino compounds tested exhibited a single irreversible oxidation peak in the region from +0.50 to +0.55 V, attributed to the Co(II)PC → Co(III)PC transition occurring in this potential region.[6] Ephedrines exhibited a distinct electrochemical behaviour, all of them being more easily oxidised than the other amino compounds. Their voltammograms at the CoPC/CPE are characterised by the presence of two anodic peaks as shown in Fig. 1. The first peak appears at +0.20 V for ephedrine and *N*-methylephedrine, and at +0.30 V for norephedrine whilst the second peak is similar to the peak observed for the other amines. Although the mechanism of the redox procedure has yet to be examined, the first peak can be attributed to the Co(I)PC → Co(II)PC transition.[6,7] Cyclic voltammograms of 1-phenylethanol and 1-phenyl-1-propanol obtained under the same conditions showed that the peak at +0.20 V cannot be attributed to the hydroxy group alone.

In all cases the total absence of cathodic return peaks indicated an irreversible electrochemical oxidation. Anodic dips during the cathodic scan, similar to those observed for cysteine and related compounds,[7] and in our previous work,[2] were present in certain cases. The peak heights were proportional to the amino compound concentration and to the square root of the scan rate, indicating a diffusion-limited process. The peak at +0.50 V suffered a severe decrease during repetitive scans and it was virtually eliminated after 5 or 6 scans. This phenomenon was expected and it has also been observed in the electro-oxidation of carbohydrates under similar conditions.[8] The electrode response was maintained by performing extended scans *versus* the cathodic direction to reach a reactivation potential of −0.30 V. This treatment slowed down the loss of sensitivity during the measurements of amino compounds, but it was not sufficient to restore the initial electrode response.

For ephedrines, we found that the electrode potential should be held at −0.30 V for at least 1 min to maintain a stable response. The ephedrine peak in the region from +0.2 to +0.3 V also suffered a decrease, strongly associated with the anodic limit of the scan, *e.g.*, by scanning up to +0.70 V, after a reactivation period of 1 min, the peak current was restored by 85%, whereas by scanning up to +0.50 V (or less) and after a reactivation period of 30 s the current was restored by 90%.

The magnitude of both oxidation peaks decreased on decreasing the pH of the electrolyte and at pH values lower than 10 no oxidation peaks were observed. Since oxidations in both acidic[7] and alkaline media[8] could be carried out with the CoPC/CPE, this observation must be attributed to the fact that amino compounds are oxidised only in relatively strong alkaline solutions. An anodic shift of nearly 60 mV pH^{-1} was observed for the ethylamine oxidation peak during the decrease of pH. A similar shift was observed for both peaks of the voltammograms of the ephedrines.

Hydrodynamic voltammetry/flow amperometry

Hydrodynamic voltammograms of 1.0 mmol L^{-1} solutions of ephedrine and ethylamine in 0.10 mol L^{-1} NaOH at both

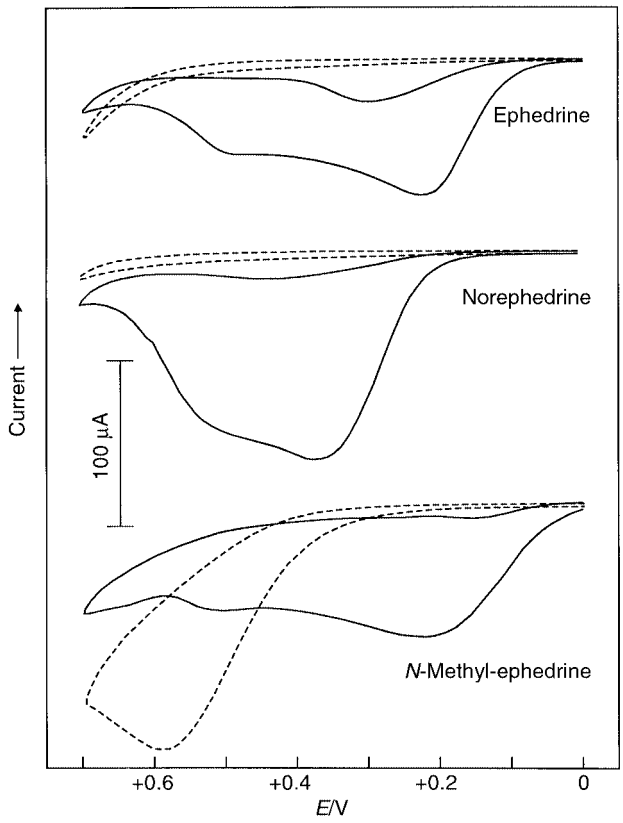

Fig. 1 Cyclic voltammograms at the CoPC/CPE (solid line) and the CPE (broken line) of 1.0 mmol L^{-1} solutions of ephedrines in 0.10 mol L^{-1} NaOH. Scan rate: 100 mV s^{-1}.

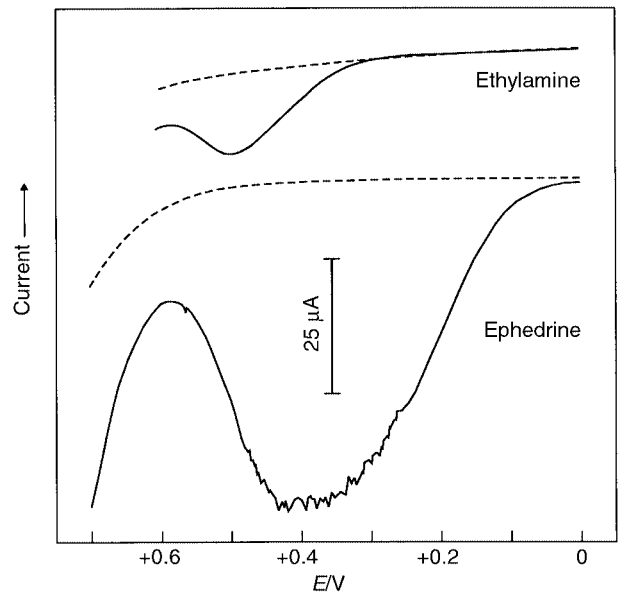

Fig. 2 Hydrodynamic voltammograms of ephedrine and ethylamine (1.0 mmol L^{-1} in 0.10 mol L^{-1} NaOH) at the CoPC/CPE (solid line) and the CPE (broken line). Scan rate: 10 mV s^{-1}.

modified and unmodified electrodes are shown in Fig. 2. The catalytic oxidation current of ephedrine reached its maximum value, under hydrodynamic conditions (constant magnetic stirring), at +0.40 V and decreased at higher potentials as a result of the electrode deactivation process occurring at such values.

Further studies of the potential dependence of the CoPC/CPE response for ephedrine were performed by measuring the peak current of repetitive injections of 1.0×10^{-4} M ephedrine at potentials varying by 50 mV steps. The peak current increased with potential increase but at values higher than +0.25 V a drift of the analytical signal occurred. Similar results were obtained with all the amino compounds tested. The deactivation process of CoPC-containing electrodes has been attributed to further oxidation and decomposition of the phthalocyanine ring, to irreversible complexation of the Co(III) centre[9] and to the gradual leaching of CoPC from the electrode surface.[2,6]

The stability of the CoPC/CPE response was markedly improved by applying a potential alternating between a cathodic reactivation potential and an anodic detection potential, as proposed by Santos and Baldwin.[8] The optimum potential waveform for ephedrine was: reactivation potential: −0.30 V for 100 ms, detection potential: +0.30 V for 220 ms. An anodic detection potential of +0.45 V was required for the other amino compounds tested. The current was sampled during the last 20 ms of the detection pulse. This waveform gave stable signals at about 60% of the analytical signal obtained when a steady detection potential of the same magnitude was applied. This signal decrease can be attributed to kinetic reasons. This reactivation approach failed to restore the electrocatalytic activity of the CoPC/CPE at detection potentials more anodic than +0.45 V, even by prolonging the reactivation pulse. Typical stability results obtained by consecutive measurements of the same ephedrine solution are shown in Fig. 3. By applying constant detection potentials a gradual loss of sensitivity always occurred. A relatively stable response was obtained by applying a constant detection potential of +0.20 V, but the S/N was markedly improved by applying the alternating potential waveform.

In all cases the analytical signal increased on increasing the alkalinity to 1.0 M NaOH. No analytical signal was observed for solutions of pH 10.0 or less. Under the flow conditions selected for the amperometric measurements, the injection current peak represented 65–70% of the steady-state signal.

Analytical characteristics and applications

The overall reproducibility of the CoPC/CPE response after renewal of the electrode surface was within 6–8%, whereas the

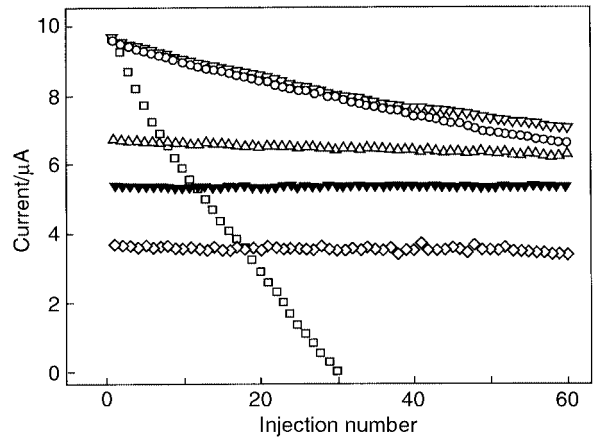

Fig. 3 Time dependence of the CoPC/CPE response for a 100 μmol L^{-1} ephedrine solution in 0.10 mol L^{-1} NaOH. Constant potential detection at: ◇, +0.20 V; Δ, +0.25 V; ▽, +0.30 V; ○, +0.35 V; and □, +0.40 V. Pulsed potential detection at: ▼, +0.30 V.

Table 1 Summary of the analytical characteristics for the flow injection determination of ephedrines and of various amino compounds by pulse amperometric detection[a]

	Analytical characteristics (regression equation data)				
Amino compound	Linear range/μM	Slope/ nA μM^{-1}	Intercept/ nA	r	Detection limit/μM[b]
Ephedrines—					
Ephedrine	1–100	54.8	3.0	0.9998	0.8
Norephedrine	1–100	57.1	3.1	0.9998	0.8
N-Methylephedrine	2–100	52.0	3.5	0.9998	1.1
Amines[c]—					
Ethylamine	10–200	15.1	1.3	0.9994	8.0
Ethylenediamine	8–200	19.3	1.8	0.9991	6.3
Benzylamine	10–200	16.2	1.8	0.9991	7.5
1-Phenylethylamine	10–200	15.9	1.6	0.9994	7.6
Amino acids[d]—					
Alanine	10–200	14.7	1.3	0.9993	8.2
Valine	10–200	14.7	1.3	0.9993	8.2
Leucine	10–200	15.1	1.2	0.9995	8.0
Isoleucine	10–200	15.3	1.5	0.9992	7.9
Phenylalanine	10–200	14.9	1.2	0.9995	8.2
Methionine	10–200	13.2	1.2	0.9992	9.2
Asparagine	20–200	7.9	1.1	0.995	15
Aspartic acid	20–200	6.9	1.0	0.997	18
Glutamine	20–200	6.3	0.9	0.998	19
Glutamic acid	20–200	7.6	0.9	0.996	16
Lysine	5–200	27.1	1.8	0.9996	4.5
Arginine	5–100	33.6	2.1	0.9992	3.6
Histidine	8–200	21.8	1.8	0.9991	5.6

[a] Anodic detection pulse: +0.30 V for ephedrines (+0.45 V for the other amino compounds) for 220 ms, cathodic reactivation pulse: −0.30 V for 100 ms (all potentials are *versus* SCE). Carrier electrolyte: 0.10 M NaOH. [b] Defined as the concentration corresponding to $3s_a$, where s_a is the standard deviation of the intercept. [c] The following amines gave detection limits in the range of 60–90 μM but no linear range covering at least one decade of concentration could be obtained under the aforementioned conditions: dimethylamine, pyrrolidine, piperidine, morpholine, ethanolamine, diethanolamine. [d] The following amino acids gave a detection limit of 70 μM but no linear range covering at least one decade of concentration could be obtained under the aforementioned conditions: serine, threonine, proline, hydroxyproline.

Table 2 Effect of various interferents (I) on the flow-amperometric analytical signal (P: current peak height) of 1×10^{-5} M ephedrine (E)

	Analytical signal ratio; $P_{E+I}:P_E$			
	[I]:[E] = 5		[I]:[E] = 10	
Detection potential	+0.30 V	+0.25 V	+0.30 V	+0.25 V
Interferent, I—				
Glucose	1.06	1.04	1.20	1.14
Lactose	1.04	[a]	1.09	1.06
Galactose	1.04	[a]	1.10	1.06
Sucrose	1.04	[a]	1.09	1.07
Sorbitol	1.06	1.05	1.23	1.15

[a] Ratio within the reproducibility range of the measurements (0.97–1.03).

Table 3 Determination of ephedrine in pharmaceutical preparations[a]

Preparation	Present method; calibration graph (n = 5)	Spectrophotometry (n = 5)
Nasal Drops (1%), Ephedrine Vogen Laboratories (Limassol, Cyprus)	(0.98 ± 0.02)%	(0.97 ± 0.02)%
Tablets (25 mg), Ephedrin Slovakofarma (Hlohovec, Slovakia)	25.6 ± 0.8 mg	25.1 ± 0.6 mg
Injection solution (50 mg), Ephedrin Biotika (Slovenska Lupca, Slovakia)	50 ± 1 mg	49 ± 1 mg

[a] Standard deviations were calculated from five individual determinations on each sample by each method.

reproducibility of consecutive measurements of 0.1 mmol L^{-1} ephedrine solutions was 2–3%. The linear range (1–100 μmol L^{-1}) and the detection limit for ephedrine (0.8 μmol L^{-1} or 0.13 μg mL^{-1}) compare favourably with those obtained with other voltammetric methods.[10–12] The calibration data for the flow injection-pulse amperometric determination of ephedrines and of various amino-compounds are given in Table 1.

All electroactive species participating in electrode reactions at the detection potentials are potential interferents. In alkaline solutions carbohydrates and polyols are also electroactive, their interference being only slightly reduced by decreasing further the detection potential. The results of an interference study on the determination of ephedrine are summarised in Table 2.

Ephedrine determination in pharmaceutical preparations

The pharmaceutical preparations used were nasal drops, injection solutions and tablets. Nasal drops and injection solutions were analysed after dilution with 0.10 M NaOH to an approximate final concentration of about 10^{-5} M. Tablets were pulverised, then mixed with the appropriate amount of ethanol. The supernatant was filtered and diluted as before. The analytical results are summarised in Table 3. The results were in good agreement with those obtained by the standard spectrophotometric method.[13]

Conclusions

The CoPC/CPE, coupled with the alternating potential technique, can serve as a sufficiently stable and very sensitive amperometric detector for the determination of ephedrines and other organic amino-compounds. Owing to the significant reduction of the anodic overpotentials, CoPC-modified electrodes can be used as working electrodes for the electrochemical detection of a wide spectrum of organic compounds, normally considered as non-electroactive. Clearly, further work is needed in order to find more effective ways for the incorporation of CoPC or other electrocatalysts of a similar nature in the working electrode matrix so as to enhance the compatibility of these electrodes with HPLC systems.

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