New insights into the oxidation pathways of apomorphine

Jorge M. P. J. Garrido,[a] Cristina Delerue-Matos,[a] Fernanda Borges,*[b] Tice R. A. Macedo[c] and Ana M. Oliveira-Brett[d]

[a] *CEQUP/Departamento de Engenharia Química, Instituto Superior de Engenharia do Porto, Rua S. Tomé, 4200-485 Porto, Portugal*
[b] *CEQOFFUP/Departamento de Química Orgânica, Faculdade de Farmácia, Universidade do Porto, 4050-047 Porto, Portugal. E-mail: fborges@ff.up.pt; Fax: +351222003977; Tel: +351222078952*
[c] *Faculdade de Medicina, Departamento de Farmacologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal*
[d] *Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade de Coimbra, 3004-535 Coimbra, Portugal*

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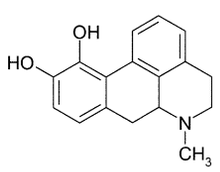
A detailed study of the oxidative behaviour of apomorphine in aqueous media is reported. Resorting to the synthesis of apomorphine derivatives it was possible to identify all the anodic oxidation peaks of apomorphine, which are related to the oxidation of the catechol and tertiary amine groups. These findings were revealed to be important since they could lead to a better understanding of the biological interactions of apomorphine and gain insight into its metabolic pathways. During the voltammetric studies, it was also found that apomorphine forms a complex with borate through the catechol group leading to an increase of its oxidation potential. This property could be very useful with regard to the stabilization of apomorphine solutions since it could drastically reduce its autoxidation.

Introduction

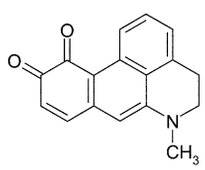
Apomorphine has been known as an emetic agent for over a century. Nevertheless, due to its dopaminergic activity it has received renewed attention because it was found to have beneficial effects in the treatment of idiopathic Parkinson's disease, which is related to the progressive degeneration of the central nervous system. In addition to Parkinson's disease, recent studies have indicated potential new uses for apomorphine, namely in the treatment of erectile dysfunction.[1] However, its inherent instability complicates its application in clinical practice. In fact, aqueous solutions of apomorphine undergo spontaneous oxidative decomposition turning green in the presence of light and air. These findings, together with reports of large inter-patient variability in apomorphine pharmacokinetics, have led to an increase of studies regarding interactions of the drug and its metabolites with plasma and tissues. The pharmacokinetic profile of apomorphine could be influenced by covalent interactions through its catechol moiety, which after oxidation gives an electrophilic quinone that can also interact with proteins and other tissue components.[2] Moreover, it is known that quinones represent a class of toxicological intermediates, which can lead to a variety of hazardous effects *in vivo*, including acute cytotoxicity, immunotoxicity and carcinogenesis.[3] The mechanism by which quinones cause these effects is quite complex and not well established. It is worth noting that the presence of an *N*-methyl group in apomorphine that could be oxidized may also influence its biological activity, since it is known that a change in the *N*-methyl group could result in a significant decrease in the affinities to 5-HT_{1A} receptors.[4]

A better understanding of the biological interactions and the metabolic pathways of apomorphine could be achieved through detailed knowledge of its electrochemical oxidation behaviour.

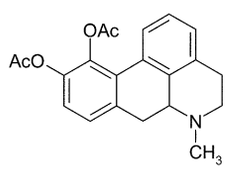
The knowledge that apomorphine metabolites could be involved in coupling reactions with nucleophilic groups of proteins,[2] together with the possibility that other oxidation products, besides oxoapomorphine,[5] could be formed by oxidation has led us to undertake a detailed study of the electrochemical oxidation behaviour of apomorphine. In order to identify all the oxidation processes and the products formed the syntheses of two apomorphine derivatives, oxoapomorphine and diacetylapomorphine (Fig. 1), were carried out as well as studies of their electrochemical behaviour.



Apomorphine



Oxoapomorphine



Diacetylapomorphine

Fig. 1 Structural formulae of apomorphine and its derivatives.

Experimental

Apparatus

All experiments were performed using a 663 VA Metrohm cell containing a glassy carbon working electrode (Metrohm, d = 2.0 mm), a glassy carbon rod counter electrode (Metrohm) and a Ag/AgCl (3 M KCl) reference electrode (Metrohm) attached to a Autolab PSTAT 10 potentiostat/galvanostat running with model GPES (EcoChimie, Netherlands). Experiments were

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made without deaeration of the solutions. A Metrohm E520 pH-meter and a glass electrode of the same make were used for the pH measurements.

Melting points were measured using a Köfler microscope. ^{1}H and ^{13}C NMR (^{1}H decoupled) spectra were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Chemical shifts are expressed as δ (ppm) values relative to tetramethylsilane (TMS) as internal reference and coupling constants (J) are given in Hz; DMSO-d_6 was used as the sample solvent. Assignments were also made from DEPT (distortionless enhancement by polarization transfer) (see underlined values). Electron impact mass spectra (EI-MS) were recorded on a VG AutoSpec instrument and data are reported as m/z (% of relative intensity of the most important fragments).

Thin layer chromatography (TLC) was carried out on aluminium sheets precoated with silica gel 60 F254 with layer thickness 0.2 mm (Merck). The following chromatographic systems were used chloroform–methanol–diethylamine (8 : 1.5 : 0.5), ethyl acetate–hexane (9 : 1). The spots were visualised by UV detection (254 nm) and iodine vapour. Solvents were evaporated in a Büchi Rotavapor. Petroleum ether used was in the boiling range 40–60 °C.

Reagents and solutions

Apomorphine was obtained from Sigma as the hydrochloride and was used without further purification. All chemicals and solvents were reagent grade and were used as received. Deionised water with conductivity less than 0.1 μS cm^{-1} was used throughout. Buffer solutions employed were 0.2 M in the pH range 1.2–12.2.[6]

Synthesis of oxoapomorphine

Using the method of Linde and Ragab.[7] An aqueous solution of apomorphine hydrochloride (250 mg) was added to 25 mL of 5% $HgCl_2$ and 50 mL of 0.2 M citric acid–phosphate buffer (pH 6). The solution was warmed at 70 °C for 30 min. After cooling, green crystals were obtained and filtered. It was found by TLC that the reaction was not complete since some starting material was present.

Using the ultrasound method. A mixture of apomorphine hydrochloride (100 mg) and silver oxide (370 mg) in ethanol was subjected to ultrasonication for 4.5 h, at 25 °C. The mixture was filtered and the solvent evaporated. The crude product was purified using flash chromatography (FC): silica gel 60 (0.040–0.063 mm, Merck) and ethyl acetate–hexane (9 : 1). Fractions containing the main product were combined and recrystallized from ethanol to give oxoapomorphine as blue-green crystals (90%; mp > 300 °C). ^{13}C NMR: 183.3 (*C*=O), 176.0 (*C*=O), 152.7, 147.2, 139.7, 134.7, 133.3, 131.5 (*C*H), 129.5, 128.7 (*C*H), 124.3 (*C*H), 123.7 (*C*H), 119.8, 108.0 (*C*H), 49.9 (CH_2), 40.1 (N–CH_3), 28.2 (CH_2); EI-MS: m/z 265 ([M + 2]$^{+\bullet}$, 76), 264 ([M + 1]$^{+\bullet}$, 19), 263 ([M]$^{+\bullet}$, 25), 235 ([M − CO]$^{+\bullet}$, 100), 206 (5).

Synthesis of diacetylapomorphine

Apomorphine hydrochloride (250 mg) was dissolved in a mixture of 1.5 mL of dry pyridine and 15 mL of acetic anhydride and maintained with stirring at room temperature, during 24 h. After reaction the solution was poured into a beaker containing ice. The crude product was extracted with chloroform (3 × 20 mL). The combined organic phases were washed with water (20 mL) and dried with anhydrous sodium sulfate. After evaporation of the solvent, a residue was obtained and recrystallized with diethyl ether and petroleum ether. Diacetylapomorphine was obtained as white crystals (88%, mp 127–128 °C). ^{13}C NMR: 168.4 (CH_3*C*=O), 168.0 (CH_3*C*=O), 141.9 (*C*OCOCH$_3$), 139.0 (*C*OCOCH$_3$), 135.1, 135.0, 133.6, 129.6, 128.9, 127.9, 126.6, 126.1, 124.1, 122.1, 65.1, 52.2 (CH_2), 43.6 (N–CH_3), 33.7 (CH_2), 28.6 (CH_2), 20.5 (CH_3), 20.4 (CH_3); EI-MS: m/z 351 ([M]$^{+\bullet}$, 62), 308 ([M − *C*OCH$_3$]$^{+\bullet}$, 67), 266 ([M − COCH$_3$]$^{+\bullet}$, 100), 250 (9), 224 (26), 206 (13), 165 (12), 152 (9).

Results and discussion

Although in aqueous solutions the degradation of apomorphine is a very rapid process accelerated with increasing pH, little is known about its mechanism. The study of the oxidative degradation mechanism of apomorphine and identification of all the electrochemical oxidation data obtained was only possible after the synthesis of the apomorphine derivatives oxoapomorphine and diacetylapomorphine (Fig. 1). The oxoapomorphine contains the *ortho*-quinone group formed during the autoxidation of apomorphine and enables the clarification of the oxidative behaviour of the catechol of apomorphine. In the compound diacetylapomorphine the catechol group is blocked by acetyl groups, consequently preventing oxidation and even the formation of oxoapomorphine. The study of the electrochemical oxidation of diacetylapomorphine enables the identification of the peak related to the oxidation of the tertiary amine present in apomorphine.

Synthesis of the apomorphine derivatives oxoapomorphine and diacetylapomorphine

The synthesis of oxoapomorphine was based on the procedure of Linde and Ragab[7] which uses mercuric chloride as the oxidant reagent. Since the use of mercury is nowadays problematic for environmental reasons, a new synthetic procedure based on the use of silver oxide and ultrasound[8] was developed in this work, and is described in the Experimental section. Diacetylapomorphine was synthesised by the classic method of acetylation (see Experimental section).

The ^{13}C NMR spectrum of oxoapomorphine confirmed the presence of aromatic, methylene, methine and methyl carbons and supported the occurrence of carbonyl functional groups at δ 183.3 and 176.0 ppm. The absence of the signals at δ 61.2 and 51.1 ppm in DEPT data confirmed the presence of a double bond between C-6a and C-7 [^{13}C NMR data of apomorphine: 145.0 (*C*–OH), 143.2 (*C*–OH), 132.3, 129.9, 128.7, 127.4 (*C*H), 126.9 (*C*H), 126.7 (CH), 124.5, 119.7, 118.5 (*C*H), 114.4 (*C*H), 61.2 (*C*H), 51.1 (CH_2), 40.9 (N–CH_3), 30.5 (CH_2), 25.5 (CH_2)]. The mass spectrum of the quinone is in accordance with the proposed structure showing two characteristic features of this class of compounds: the loss of carbon monoxide and the formation of a peak with two mass units higher than the molecular weight.[9] Based on the spectral data the structure of oxoapomorphine was proposed as shown in Fig. 1, which is in accordance with that anticipated by Erhardt *et al.*[10] The proton NMR spectrum was identical to that reported by Linde and Rabad.[7] It is noteworthy that the oxidant agent employed in this work was not the reagent described in the literature for the synthesis of the compound.[7]

The chemical identity of diacetylapomorphine (Fig. 1) was established by NMR and MS. The presence of signals in the ^{13}C NMR spectrum at δ 168.4 (CH_3*C*=O), 168.0 (CH_3*C*=O), 141.9 (*C*OCOCH$_3$), 139.0 (*C*OCOCH$_3$) and 20.5 (CH_3), 20.4 (CH_3) and the absence of signals in the ^{1}H NMR spectrum at δ 9.8 and 8.8 (C–O*H*) and the presence of two acetyl groups at δ 2.5 and 2.3 led to the structural elucidation of this compound. The mass spectrum of the compound is in agreement with the proposed structure.

Effect of pH

The anodic oxidation of apomorphine is a complex mechanism which is pH dependent and three oxidation processes can be observed. As will be demonstrated below these processes could be identified after studying the electrochemical oxidation of the

synthesised apomorphine derivatives oxoapomorphine and diacetylapomorphine (Fig. 1).

The electrochemical behaviour of apomorphine was studied over a wide pH range, between 1.2 and 12.2, at a glassy carbon working electrode using differential pulse voltammetry (Figs. 2 and 3). The first peak (I) in Fig. 2, in very acid media,

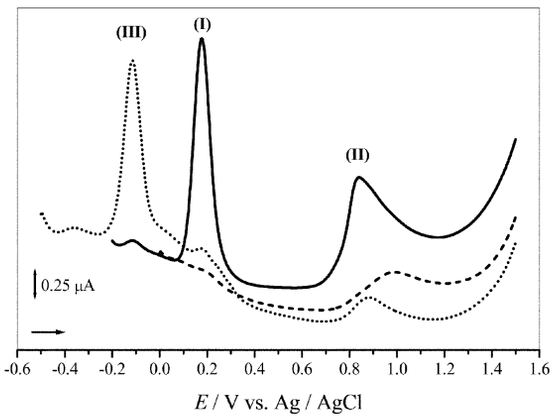


Fig. 2 Differential pulse voltammograms in pH 6 of 0.1 mM solutions: (—) apomorphine, (· · ·) oxoapomorphine and (---) diacetylapomorphine in 0.2 M ionic strength phosphate buffer. Scan rate 5 mV s^{-1}.

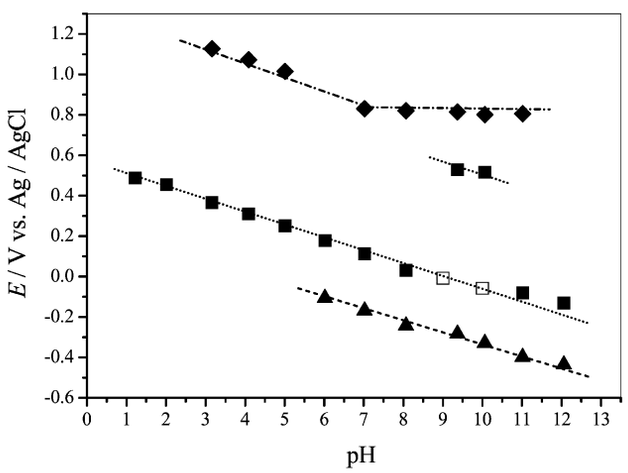
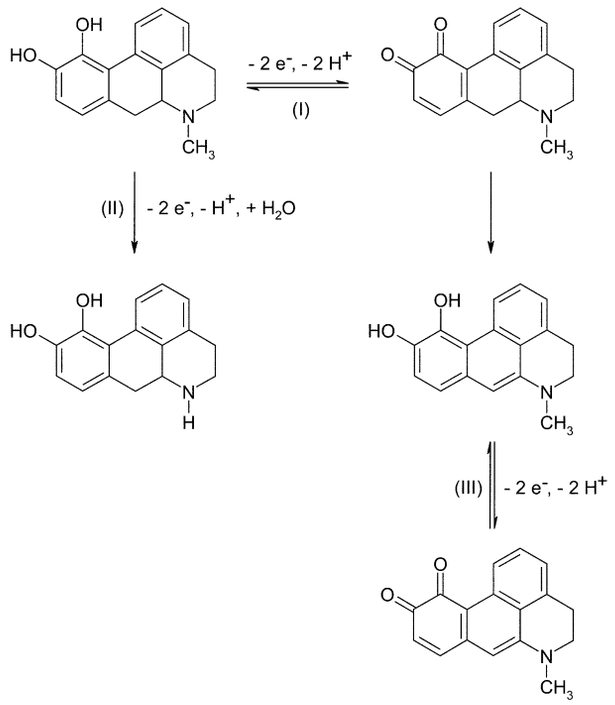


Fig. 3 Plot of E_p *vs.* pH from differential pulse voltammograms of 0.1 mM apomorphine in 0.2 M ionic strength buffer electrolyte. For pH 9 and 10 results are presented using borate buffers (■) and ammonia buffers (□). Scan rate 5 mVs^{-1}. Slope 59.2 mV per pH unit.

corresponds to the oxidation of catechol, the only peak starting at pH 1, $E_p = +0.5$ V. The second peak (II), starting at pH 3, $E_p = +1.1$ V, corresponds to the oxidation of the tertiary amine. The third peak (III), starting at pH 6, $E_p = -0.1$ V, corresponds to the oxidation of the species in equilibrium with oxoapomorphine, occurring as a result of spontaneous homogenous oxidative decomposition of apomorphine to oxoapomorphine (Scheme 1).

The anodic wave observed starting at pH 1, $E_p = +0.5$ V, the peak potential decreasing as the pH increases, was easily attributed to the oxidation of the catechol group after the voltammetric behaviour of the apomorphine derivatives, oxoapomorphine and diacetylapomorphine was studied. In both compounds the absence of the apomorphine oxidation peak at +0.5 V in acid media (Figs. 4 and 5) was observed. These results led to the conclusion that this peak is related to the oxidation of the catechol group present in apomorphine giving oxoapomorphine. Moreover, this result is also in agreement with the data found in the literature, which gives oxoapomorphine as the only oxidation product of apomorphine.[5]

By studying the oxidation behaviour of diacetylapomorphine (Fig. 4) it was possible to identify the apomorphine oxidation



Scheme 1 Oxidation mechanism of apomorphine.

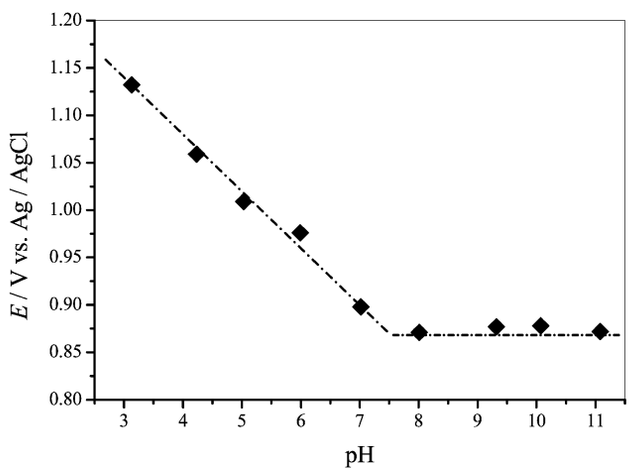


Fig. 4 Plot of E_p *vs.* pH from differential pulse voltammograms of 0.1 mM diacetylapomorphine in 0.2 M ionic strength buffer electrolyte. Scan rate 5 mV s^{-1}. Slope 59.2 mV per pH unit.

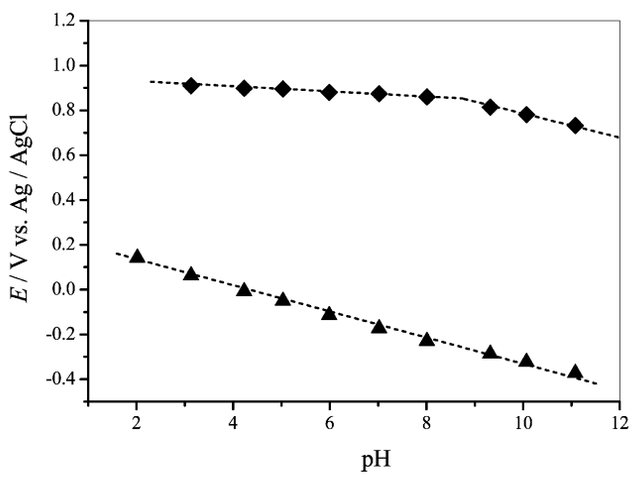


Fig. 5 Plot of E_p *vs.* pH from differential pulse voltammograms of 0.1 mM oxoapomorphine in 0.2 M ionic strength buffer electrolyte. Scan rate 5 mV s^{-1}. Slope 59.2 mV per pH unit.

peak starting at pH 3, E_p = +1.1 V. This peak is due to the oxidation of the tertiary amine group present in the apomorphine molecule. This was rapidly confirmed because in diacetylapomorphine the catechol position is blocked by acetyl groups (Fig. 1) and presents only one anodic wave, initially appearing at +1.1 V and decreasing in potential as the pH increased (Fig. 4). The oxidation of the tertiary amine led to the formation of a secondary amine and an aldehyde.[11] These results undoubtedly show that the second peak of apomorphine, starting at pH 3, E_p = +1.1 V, is due to the oxidation of the tertiary amine group present in the apomorphine molecule (Fig. 3) and not to further oxidation of oxoapomorphine. In aqueous solution apomorphine rapidly undergoes spontaneous oxidative decomposition to oxoapomorphine, this process being accelerated by oxygen and high pH.[5] The apomorphine oxidation wave starting at pH 6, E_p = −0.1 V, could be related to an oxidation process involving the catechol group of the intermediate (Scheme 1) generated *in situ* in oxoapomorphine solutions (Fig. 3).

Another important observation is that even at pH lower than 6 this apomorphine oxidation peak appeared if several consecutive scans were done. In fact, consecutive scans showed the appearance in the second scan of the oxidation peak at −0.3 V and growth of the peak occurred during the following scans while the apomorphine oxidation peak at +0.5 V does not increase with time. This could be easily explained by the ability of the new redox couple, formed by oxidation of the catechol group of apomorphine (see Scheme 1), to adsorb very strongly on the solid electrodes[5] and to the reversibility of the oxoapomorphine peak occurring at −0.3 V, clearly shown in the square wave voltammogram of Fig. 6. The square wave voltammogram

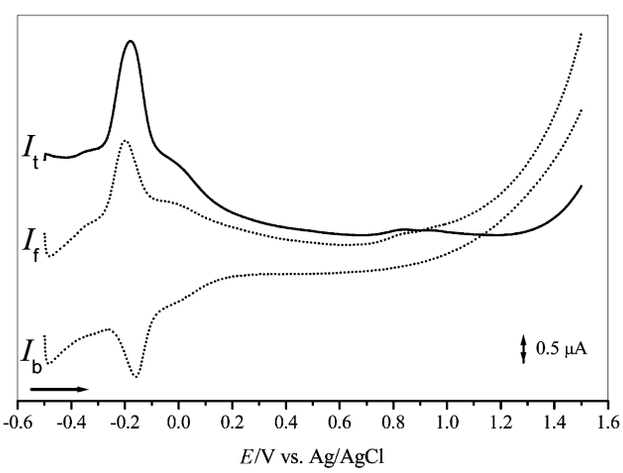


Fig. 6 Square-wave voltammogram of 0.1 mM oxoapomorphine at pH 7 with 0.2 M ionic strength buffer electrolyte. ($I_t = I_f - I_b$, I_t = total current, I_f = forward current and I_b = backward current). Frequency 150 Hz; pulse amplitude 50 mV.

shows similar values for the forward and backward current and peak potentials, characteristic of reversible electron transfer reactions. The most relevant data regarding this oxoapomorphine oxidation peak comes from the electrochemical study of oxoapomorphine with pH (Fig. 5). The oxidation behaviour of oxoapomorphine studied by differential pulse voltammetry showed an anodic peak, over the whole pH range, at the same oxidation potential as the peak appearing in a solution of apomorphine. This is very good evidence for the fact that the apomorphine oxidation wave starting at pH 6, E_p =−0.1 V, is related with the above-mentioned oxidative process (see Scheme 1).

Effect of supporting electrolyte

During the apomorphine voltammetric study over a wide pH range a very interesting aspect of its oxidation mechanism not studied so far was observed which has important implications on solution stability. As mentioned earlier aqueous solutions of apomorphine undergo spontaneous oxidative decomposition turning green in the presence of light and air and its instability deters application in clinical practice.

Comparing the oxidation patterns of apomorphine for pH 8, 9, 10 and 11 in Fig. 3, it is surprising to observe a drastic change for pH 9 and 10. In fact, for these pH values not only did the peak around 0.0 V, corresponding to oxidation of the catechol group disappear, but a new peak also appeared at +0.5 V (Fig. 7). This clearly indicates the occurrence of an interaction between the catechol group in apomorphine and the supporting electrolyte.

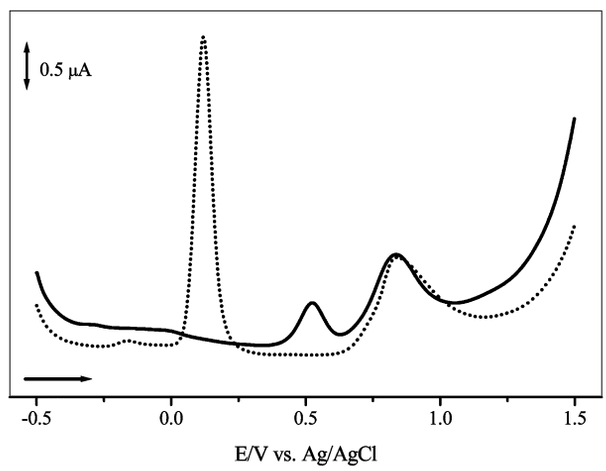


Fig. 7 Differential pulse voltammograms of 0.1 mM apomorphine at pH 9 with 0.2 M ionic strength buffer: (—) borate and (···) ammonia. Scan rate 5 mV s^{-1}.

At pH 9 and 10 the supporting electrolyte used was borate buffer. In the literature the formation of complexes between boric acid or borate and catechols or substituted catechols is described,[12,13] which easily explains the shift in the oxidation potential of the complexed catechol to more positive values. Nevertheless, to prove that this interaction was due to the composition of the buffer used, the voltammetric oxidation of apomorphine was studied again but now using ammonia buffer at pH 9 and 10. The results obtained (Fig. 3) fit perfectly to the line of E_p *vs.* pH. This clearly shows that the oxidation peak for the apomorphine catechol group is at the potential of +0.1 V in ammonia buffer, whereas the oxidation of the catechol group in the apomorphine–borate complex is at a much higher potential of +0.5 V, in borate buffer. This finding suggests that the use of borate buffer for storage of apomorphine would prevent autoxidation of apomorphine in an aqueous stock solution, due to the increase of the apomorphine–complex oxidation potential. The implication that stabilisers are not needed in apomorphine–borate buffer solutions[14,15] means a widening of possible applications in clinical practice.

Conclusions

The oxidation mechanism of apomorphine in aqueous solution was found to be complex and strongly pH dependent; a detailed study of the oxidation behaviour of apomorphine and its derivatives with pH was performed. The synthesis of the apomorphine derivatives, oxoapomorphine and diacetylapomorphine, was undertaken and enabled the identification of all the anodic peaks found for the apomorphine oxidation and verified that they are related to the oxidation of catechol and tertiary amine groups. This could lead to important advances in understanding the metabolic pathways and biological interactions of apomorphine. Moreover it was found

that apomorphine forms a complex with borate through the catechol group which has a higher oxidation potential than in ammonia buffer. This means improved apomorphine stabilization in borate buffer solutions, due to the increase of the apomorphine–complex oxidation potential, and suggests the use of these conditions for storage without the need for stabilisers.

Acknowledgements

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References

1 H. Padma-Nathan and F. Giuliano, *Urol. Clin. North Am.*, 2001, **28**, 321.
2 R. V. Smith, R. B. Velagapudi, A. M. McLean and R. E. Wilcox, *J. Med. Chem.*, 1985, **28**, 613.
3 J. L. Bolton, M. A. Trush, T. M. Penning, G. Dryhurst and T. J. Monks, *Chem. Res. Toxicol.*, 2000, **13**, 135.
4 M. H. Hedberg, J. M. Jansen, G. Nordvall, S. Hjorth, L. Unelius and A. M. Johansson, *J. Med. Chem.*, 1996, **39**, 3491.
5 H.-Y. Cheng, E. Strope and R. N. Adams, *Anal. Chem.*, 1979, **51**, 2243.
6 A. M. Oliveira-Brett, M. M. M. Grazina, T. R. A. Macedo, C. Oliveira and D. Raimundo, *J. Pharm. Biomed. Anal.*, 1993, **11**, 203.
7 H. H. A. Linde and M. S. Ragab, *Helv. Chim. Acta*, 1968, **51**, 683.
8 C. A. Hall III, S. L. Cuppett and P. Dussault, *J. Agric. Food Chem.*, 1998, **46**, 1303.
9 K.-P. Zeller, in *The Chemistry of the Hydroxyl Group: Part 1*, ed. S. Patai, Interscience, London, 1971, pp. 231–255.
10 P. W. Erhardt, R. V. Smith, T. T. Sayther and J. E. Keiser, *J. Chromatogr.*, 1976, **116**, 218.
11 J. R. L. Smith and D. Masheder, *J. Chem. Soc., Perkin Trans. 2*, 1977, 1732.
12 K. Yoshino, M. Kotaka, M. Okamoto and H. Kakihana, *Bull. Chem. Soc. Jpn.*, 1979, **52**, 3005.
13 R. Pizer and L. Babcock, *Inorg. Chem.*, 1977, **16**, 1677.
14 E. Sam, P. Augustijns and N. Verbeke, *J. Chromatogr., B: Biomed. Appl.*, 1994, **658**, 311.
15 R. Van der Geest, P. Kruger, J. M. Gubbens-Stibbe, T. Van Laar, H. E. Bodde and M. Danhof, *J. Chromatogr., B: Biomed. Appl.*, 1997, **702**, 131.