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The study of the differential pulse voltammetric behaviour of ergot alkaloids and their determination by DC amperometric detection in a FIA system*

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Abstract: The ergot alkaloids possess strong pharmacological effects and are important drugs with widespread clinical uses. The ergot alkaloid preparations manufactured by Gedeon Richter Chemical Works Ltd have a very low active substance content (e.g. 1.0 mg in each Secadol (0.4 g) coated tablet); therefore a sensitive method of determination must be used. Because of this constraint the differential pulse voltammetric behaviour of ergot alkaloids was studied in respect of the effects of pH and composition of media and an automated FIA system with amperometric detection has been used to develop a selective and sensitive method for the routine quantitative assay of these alkaloids. A short summary is given of the experimental evidence to substantiate the stoichiometric equation proposed for the electrochemical oxidation of the lysergic acid type of ergot alkaloids, the mechanism may be generally applicable for compounds having the ergoloid skeleton. In the course of the work it was concluded that a simple DC amperometric method of detection in a FIA system could be applied to determine the content of ergot alkaloids of different pharmaceutical preparations. A suitable method designed to meet current analytical requirements has been developed and validated.

Keywords: *Electrochemical oxidation; ergot alkaloids; differential pulse voltammetry; DC voltammetry; controlled potential coulometry; automated FIA; DC amperometric detection.*

Introduction

Ergot alkaloids of the lysergic acid type have extensive therapeutic uses in the prevention of postpartum or postabortal haemorrhage and in the treatment of migraine [1]. The most important members of this type of alkaloid are ergotamine tartrate and ergonovine (ergometrine) maleate. Pharmaceutical preparations containing these ergot alkaloids are traditional products of Chemical Works of Gedeon Richter Ltd. In order to determine the content of active ingredients of these products, different methods have been developed, which are chemically independent.

Wang and Ozsoz proposed a sensitive differential pulse voltammetric (DPV) method for the determination of ergotamine tartrate, ergonovine maleate and ergocristine by applying a lipophilic membrane layer at a glassy carbon electrode [2]. Belal and Anderson proposed a flow injection (FIA) analytical method with electrochemical detection for the determination of ergotamine and ergonovine [3]; they commented that ergonovine is an amino ergot alkaloid and can be oxidized at positive potentials as can many other amines.

Work done by one of the authors of present paper (G. Farsang) has shown that the electrooxidation of ergot alkaloids is a complex process in which oxidation of the indol part of the lysergic acid skeleton is dominant. That work will be published in detail elsewhere but the main route of the oxidation of ergot alkaloids containing the lysergic acid skeleton is described later in the present paper.

Paper chromatography, TLC and HPLC have been used for the separation and quantitative analysis of these alkaloids; the methods used for detection were UV spectrophotometry, densitometry, fluorimetry and fluorodensitometry [4–8].

The main aim of the present work was to develop a sensitive routine method for the measurement of products containing ergot alkaloids without any previous separation from

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the excipients. The electrochemical behaviour of ergotamine tartrate and ergonovine maleate as well as the ergotoxine alkaloids has been studied with the objective of developing a rapid and accurate automated FIA analytical method for routine analysis.

Experimental

Instrumentation

The linear sweep DC and differential pulse voltammetric studies were carried out with a Metrohm 626 Polarecord polarograph. The electrochemical cell contained a glassy carbon working electrode (Model 6.1204.040), an Ag/AgCl/c(KCl):3 M reference electrode (Model 6.0711.000) and a platinum auxiliary electrode (Model 6.0331.000), all made by Metrohm (Herisau, Switzerland).

In the electrochemical investigation of the lysergic acid type of ergot alkaloids, cyclic voltammetry and controlled potential coulometry have been used. For cyclic voltammetry of ergot alkaloids, a Bruker E-100 type universal polarograph (USA) and a Linseis LY 1700 *x–y* recorder were used. The potentiostat input of that polarograph was driven by a signal generator made by Elektroflex (Szeged, Hungary).

A Tacussel ASA 4-SHT2 type potentiostat combined with an IG 5N type integrator (Lyon, France) was used to carry out controlled potential coulometry (CPC). The home-made preparative electrolysis cell contained a cylindrical platinum net working electrode and a platinum cylinder counter electrode in a glass tube filled with supporting electrolyte separated from the bulk solution by a glass filter of G-3 porosity (Schott, Jena, Germany); the cell also contained a Tacussel RDJ 10 Ag B10 double junction reference electrode containing 10^{-2} M $AgNO_3$ and the salt bridge was filled with 10^{-1} M $LiClO_4$ dissolved in acetonitrile. During the CPC electrolysis the electrolysed solution was stirred by a magnetic bar driven by a magnetic stirrer and N_2 gas (purity 99.99%) was bubbled through the solution by a thin glass tube.

The molecular weight of the isolated main oxidation product was determined by measuring the mass number of the molecular peak by mass spectrometry with a Kratos MS 80 type instrument using a desorption chemical ionization method.

The FIA system used was built up from the following parts. A commercial three-electrode amperometric thin-layer cell (Phillips, Cambridge, UK) was used as detector, comprising a glassy carbon working electrode of 3 mm diameter, a platinum auxiliary electrode and a Ag/AgCl/0.3 M KCl reference electrode. Both the Metrohm glassy carbon electrode and the working electrode of the Phillips wall jet cell were regularly polished by alumina paste of 0.3 μm grain size (Buehler AB gamma polishing alumina no. 3, Evanston, IL, USA). A Phillips PU 4022 (Cambridge, UK) potentiostat and *I–V* converter was used. Its output voltage (1 V) was connected to a recorder in order to register the signals generated by injecting samples into the continuous carrier stream. The mobile phase was pumped through with compressed air. The automated home-made FIA system was constructed earlier and has been described elsewhere [8]. A scheme of the FIA apparatus is shown in Fig. 1. The flow rate was set at 0.5 ml min^{-1}. The whole system was controlled by an IBM PC, namely the working of the autosampler, the number and sequence of samples and standards, the sampling time and the evaluation of FIA curves.

Chemicals and reagents

Britton–Robinson buffers (0.04 M) were

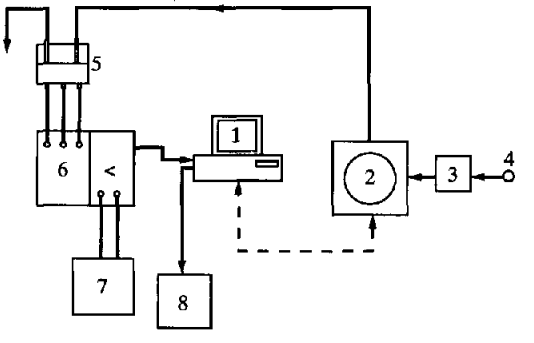


Figure 1
Scheme of the home-made automated FIA apparatus. 1, PC controlling the whole FIA system, collecting and processing the data; 2, carousel sample holder, with the injector and the container of carrier solution; 3, pneumatic pressure controller built from logic circuits; 4, coupling point of gas cylinder containing pressurized air; 5, Phillips wall jet amperometric cell; 6, potentiostat and *I–V* converter (Phillips PU 4022) to polarize the amperometric cell, with high gain amplifier for *I–V* converter; 7, analogue *x–t* recorder; 8, printer for the documentation of digitized and processed signals.

prepared from equimolar acetic acid, phosphoric acid and boric acid. The pH was adjusted with 0.2 M NaOH. Solutions of ergotamine tartrate (1×10^{-4} M) and ergonovine maleate (2×10^{-4} M) were prepared daily in acetonitrile–aqueous buffer (1:1, v/v).

In the FIA system the carrier solution was acetonitrile–KH_2PO_4 buffer (pH 2, 0.02 M) (1:1, v/v). All chemicals used in FIA experiments were AR grade Merck (Darmstadt, Germany) and were used as supplied. Ergot alkaloids standards were of BP quality.

For elucidation of the oxidative electrode reaction of ergot alkaloids, 9,10-dihydroergotoxine methansulphonate and each of its three components, 9,10-dihydroergocryptine, 9,10-dihydroergochristine and 9,10-dihydroergocornine were used as supplied by Chemical Works of Gedeon Richter Ltd. Acetonitrile (Janssen Chimica) of spectrophotometric grade was used throughout the work. Anhydrous $LiClO_4$ (Fluka A.G. Buchs SG, purum) was used to prepare base electrolytes in acetonitrile after keeping it over P_2O_5 under vacuum in a desiccator for 24 h. Water-free 1 M $HClO_4$ in acetic acid was prepared by adding a calculated amount of acetic acid anhydride to the solution prepared, the solution was then warmed to 60°C, cooled to room temperature and left for 24 h before use.

The ergopeptide alkaloids, 9,10-dihydrolysergol and 2-chlorlysergol, together with 9,10-dihydrolysergic acid (DHLA) were used as supplied by Chemical Works of Gedeon Richter Ltd. The methyl ester of 9,10-dihydrolysergic acid (DHLAME) was prepared by dissolving DHLA in dry methanol and bubbling dry HCl gas through the solution; after completion of the reaction, the DHLAME was purified by an extraction method.

For TLC aluminium sheets pre-coated with silica gel 60 F254 (Merck, Darmstadt, Germany) were used; for preparative TLC RP C18 plates (Merck, Darmstadt, Germany) were used. For isolation of the electrooxidation products a glass RP C18 cartridge was used to bind the products; the products were then washed by water and redissolved in chloroform–methanol (1:1, v/v). The solvent was removed by evaporation in a rotary film evaporator and the product was dried under vacuum at room temperature.

The experiments were conducted at room temperature.

Results and Discussion

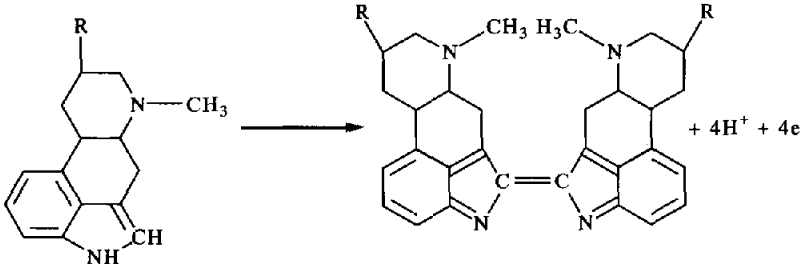
The electrochemical oxidation reaction of the lysergic acid type of ergot alkaloids

In order to elucidate the electrochemical oxidation reaction of the lysergic acid type of ergot alkaloids, cyclic voltammetric (CV) controlled potential coulometric (CPC) experiments were carried out with components of 9,10-dihydroergotoxine group, namely 9,10-dihydroergochristine, 9,10-dihydroergocornine and 9,10-dihydroergocryptine. These are ergopeptide type alkaloids, where the stability of the compounds is increased by catalytic hydrogenation of the double bond at the 9,10 positions in the ergoline skeleton. Where water was used as the solvent the filming effect resulted in poor reproducibility of the CV curves; similar effects were observed in water–acetonitrile solvent mixtures in stationary solutions. Excellent reproducibility was observed in pure acetonitrile solvent containing 0.1 M $LiClO_4$ or 0.1 M Bu_4NClO_4 as the supporting electrolyte acidified by one equivalent of percholoric acid dissolved in acetic acid. Acetic acid is an extremely weak acid in acetonitrile and its quantity is negligible when the concentration of the substance investigated is in the 10^{-3}–10^{-4} M range. All three dihydroergopeptide alkaloids gave the same CV behaviour; a single sharp irreversible anodic peak was observed on the CV curves at the same peak potential. Within the accuracy range of voltammetric potential measurements no change was observed when the CV behaviour of other ergot derivatives, like lysergic acid, 9,10-dihydrolysergic acid and its methyl ester, lysergol, 9,10-dihydrolysergol and ergotamine was studied. This result means that a part of the ergoloid structure is responsible for the oxidation process. The cyclic tripeptide part can be excluded from the oxidation process. Only the variation of the i_p values measured at the same molarity was observed for the different compounds in accordance with the different diffusion coefficients. The controlled potential coulometric experiments were carried out on all compounds mentioned earlier in the same media; the weighed amount was varied to give concentrations in the 10^{-3}–10^{-4} M range.

In all cases the originally colourless solution became deep purple (λ_{max} = 526 nm) during electrolysis.

The acid produced by electrolysis was

titrated with a strong base (piperidine or pyridine) using a microsyringe burette; the colour of the oxidized solution turned to yellow (λ_{max} = 397 nm) when two equivalents of base had been added allowing for the acid added before starting the electrolysis. Spectrophotometric measurements showed that this acid–base equilibrium is reversible. The strong colour could be explained only by the formation of a highly conjugated molecule. At the same time the product did not give the Van Urk reaction (C_2 position of the lysergic acid part is not free). The electric charge needed for the total oxidation of the weighed quantity always corresponded to a two-electron oxidation of the molecules investigated ($n_{app.}$ = 2 ± 0.1). After the isolation of the product formed by the oxidation of 9,10-dihydrolysergol, mass spectrometry gave 508 ± 1 mass number line, as the molar peak. The calculated molecular weight of the dimer oxidized form of 9,10-dihydrolysergol is 508.66. According to these results, the following stoichiometric equation is suggested for the oxidation of lysergic acid type of ergot alkaloids:



where R depends on the ergot alkaloids or ergot derivative investigated.

The experimental results present strong evidence that all those electroanalytical methods (DPV, FIA, with amperometric detection and HPLC with amperometric detection) are based on this oxidation reaction of ergoloid compounds.

Work is in progress to elucidate in detail the mechanism of the oxidative dimerization reaction discussed briefly here.

Differential pulse voltammetric (DPV) and DC voltammetric study of ergot alkaloids for FIA

The DPV voltammograms of ergotamine tartrate and ergonovine maleate were obtained in the range of pH* = 1–7. The concentration of solutions for DPV voltammetric tests was always 1 × 10^{-4} M for ergotamine tartrate or 2 × 10^{-4} M for ergonovine maleate. This concentration provided optimal concentration for the investigation of pH dependence.

At pH* >7 an opalescence was observed because of the precipitation of the alkaloid base, which set the upper limit for the measurements of pH dependence.

The peak potential decreased linearly with an increase in pH* in the range of pH* 1–7 in accordance with the electrode reaction, which takes place by producing free protons at the electrode surface. The $E_p = a + b.pH^*$ equation for the pH* dependence of peak potential E_p calculated for ergotamine tartrate (1 × 10^{-4} M) was: E_p = 1.0916 − 0.0502.pH* (a dimension = V and b dimension = V.pH*$^{-1}$), n = 13, r = 0.996. For ergonovine maleate (2 × 10^{-4} M) the equation was: E_p = 1.0555 −0.0515.pH*, n = 13, r = 0.998. In the same way the DPV peak height (I_p) decreased linearly with pH*. The calculated equation for ergotamine tartrate (1 × 10^{-4} M) was: I_p = 2.9254 − 0.3495.pH* (a dimension = μA and b dimension = μA.pH*$^{-1}$), n = 13, r = 0.998. For ergonovine maleate (2 × 10^{-4} M) the equation was: I_p = 2.5239 −0.3173.pH*, n = 13, r = 0.994.

In the course of these measurements it was observed that a highly reproducible signal can be obtained at pH* 2. At this pH* no filming effect was observed and, in addition, the current was sufficiently high. In accordance with this finding all further experiments were carried out at pH* 2.

Linear sweep voltammetric experiments showed that each of the ergot alkaloids investigated gave a single anodic peak near +1.0 V. Both the peak potentials and peak heights gave similar pH dependence to the DPV curves. Cyclic voltammetry proved that the electrode reaction is totally irreversible.

Selectivity. As can be seen in Fig. 2, the solvent and placebo do not interfere with the

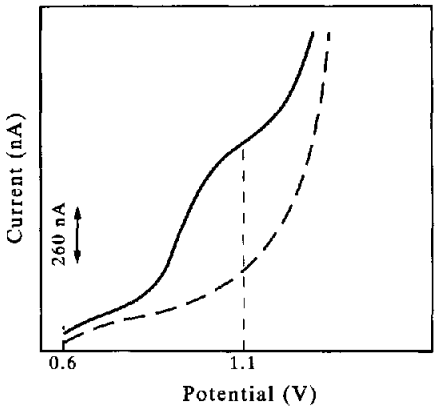


Figure 2
The DC voltammetric curves recorded for a solution of 2 μg ml^{-1} ergotamine tartrate (——) and 0.2 mg ml^{-1} caffeine (---). Acetonitrile–0.02 M KH_2PO_4 aqueous buffer (1:1, v/v, pH* = 2). Working electrode: glassy carbon.

FIA determination of the active substance at the working potential and pH* applied. The applied potential for FIA measurements +1.1 V versus the reference electrode was chosen.

Linearity. Three portions of standards were weighted into separate measuring flasks. Five aliquots from each flask were transferred into volumetric flasks. Points in the linearity study were equivalent to 50, 75, 100, 125 and 150% of the declared ergotamine tartrate content. In this range a linear relationship was obtained between the current (nA) and the concentration (μg ml^{-1}); the slope was 363 nA μg^{-1} ml, the intercept was 84.9 nA; the *F*-test results were $F_{calc.} = 2.63 < F_{theor.}$ (3.10) = 3.75.

Limit of detection and limit of quantitation. Figure 3 shows the noise level *N* and the signal

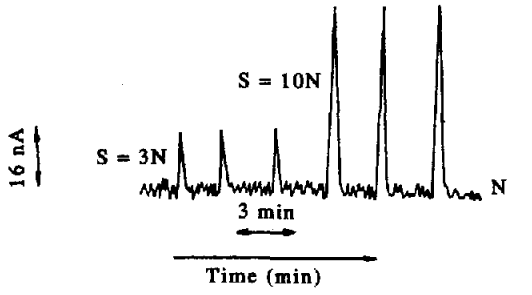


Figure 3
The determination of the limit of detection and limit of quantitation. N is the noise level of the amperometric cell in the carrier stream. S is the peak height measured after injection of ergotamine tartrate solution.

S. *S/N* = 3 was accepted as the limit of detection and *S/N* = 10 as the limit of quantitation. The limit of detection was 0.037 μg ml^{-1} and the limit of quantitation 0.12 μg ml^{-1} ergotamine tartrate.

Determination of content of active substance in a manufactured dosage form (coated tablet)

Sample preparation. For the preparation of coated tablet solution a coated Secadol tablet (0.4 g) containing 1 mg of ergotamine tartrate and 100 mg of caffeine was disintegrated by dispersion for a few min in 2 ml of distilled water; the dispersion was transferred into a 50-ml volumetric flask and diluted to 50 ml with the carrier solution. A 1 ml volume of that stock solution was transferred by pipette into a 10-ml volumetric flask and diluted to 10 ml with the carrier solution. The nominal concentration of this solution was about 2 μg ml^{-1}.

In a similar way a corresponding standard solution was also prepared.

The solutions were placed in the carousel of the autosampler and measured, after injection, with DC amperometric detection.

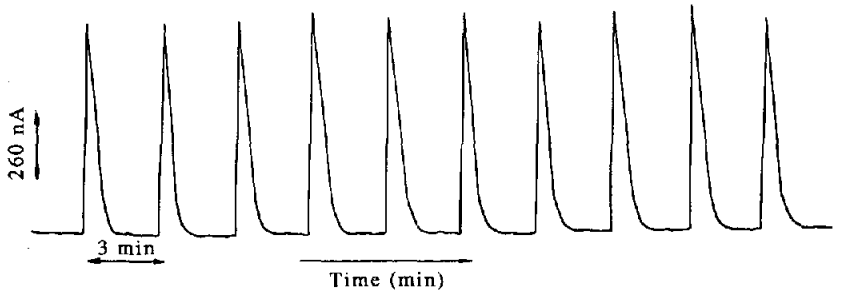


Figure 4
DC amperometric FIA curves recorded in the solutions of 10 Secadol coated tablets. The ergotamine tartrate concentration of the injected solutions prepared from individual tablets: 2 μg ml^{-1}. Carrier solution: acetonitrile–0.02 M aqueous buffer KH_2PO_4 (1:1, v/v, pH* = 2). Flow rate of carrier stream: 0.5 ml min^{-1}. Injected volume: 60 μl.

Accuracy. Five aliquots from the standard solution and standard + placebo solutions were placed into separate flasks. Concentration values set in the accuracy study were equivalent to 50, 75, 100, 125 and 150% of the declared ergotamine tartrate content. The peak current values measured in the standard solutions were plotted as a function of peak currents measured in the standard + placebo solutions. The plot gave a straight line with $tg\alpha = 1$; $r = 0.998$.

Precision. The precision of the method was measured for the concentration level corresponding to the 100% ergotamine tartrate content of the coated tablet. The RSD was 1.9%. In Fig. 4 10 FIA amperometric signals are shown; these were recorded after the injection of solutions each prepared from individual tablets.

Conclusions

A rapid, selective and accurate method based on voltammetric detection in a PC-controlled automated FIA system was developed for the determination of ergot alkaloids in solid dosage forms. This method can be used as a routine analytical procedure which meets current analytical requirements.

Acknowledgements — The preparation and purification of 9,10-dihydrolysergic acid methylester from 9,10-dihydrolysergic acid was carried out according to the advice given by Professor Henning Lund in the Department of Chemistry, University of Aarhus, Denmark, whose kind help during the stay of G. Farsang in his laboratory is gratefully acknowledged.

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