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Determination of mitoxantrone by flow injection analysis using an amperometric detector

JUAN CARLOS CORTINA VILLAR, AGUSTÍN COSTA GARCÍA and PAULINO TUÑÓN BLANCO*

Department of Physical and Analytical Chemistry, University of Oviedo, 33006 Oviedo, Principality of Asturias, Spain

Abstract: Mitoxantrone was determined by flow injection analysis using a flow cell modified in the laboratory and fitted with carbon paste as an amperometric detector. The sample solution (100 μl, 5×10^{-8}–1×10^{-5} M) was injected into the carrier stream of 0.1 M perchloric acid (pH 1.12). Mitoxantrone was determined by oxidation at the carbon paste electrode (CPE) at +0.90 V. A 60-cm delay coil (0.5 mm i.d.) was incorporated just before the detector (a canal thin layer) and a flow rate of about 4 ml min^{-1} was used. The system was successfully applied to the determination of mitoxantrone in a pharmaceutical preparation; the method was fast and reproducible.

Keywords: *Flow injection analysis; amperometric detection; mitoxantrone; mitozantrone.*

Introduction

Mitoxantrone (MXT; Fig. 1) is 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)-amino]ethyl] amino]-9,10-anthracenedione dihydrochloride. It is one of a long series of polycyclic aromatic compounds which has been shown to have antitumour activity, and has been studied because of its potential to intercalate with DNA [1–4].

Nowadays, reliable and economic methods are required for the analysis of biologically important compounds in the quality control of food and pharmaceutical products and a growing interest in the application of electroanalytical techniques has been observed [5]. The problem of analysing a large number of samples in a reasonably short time can be satisfactorily resolved if a flow injection analysis (FIA) system with electrochemical detection can be applied [6].

The electrochemical behaviour of MXT on a carbon paste electrode (CPE) has not been established. The polarographic behaviour of MXT in the presence of cuprous and cupric salts has been reported and a probable explanation of its antineoplastic activity proposed [7]. Furthermore, both electron spin resonance spectroscopy and electrochemical studies have been applied to obtain a better understanding of the metabolism of MXT [8].

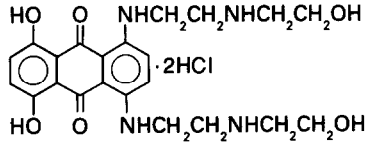

Figure 1
Structure of mitoxantrone.

MXT can be oxidized on a CPE giving two anodic processes, the first being of more interest to the analyst. In this paper the possibility of using this process for analytical purposes in a flowing system is demonstrated. Although the determination of MXT is possible using a voltammetric method on carbon paste, the inclusion of an activation step is necessary before each measurement; the purpose of this step is to eliminate adsorbed products on the working electrode. As an alternative, a FIA method is described for the determination of MXT. This technique does not require an activation step between measurements, which facilitates the development of a very fast and reliable analytical method. This method has been used to determine MXT in an aqueous solution in ampoules.

Experimental

Apparatus

Cyclic voltammetry experiments were

* Author to whom correspondence should be addressed.

carried out using a Metrohm E-611 potentiostat coupled to a Metrohm E-612 scanner. Voltammograms were recorded on a Graphtec WX-4421 X–Y recorder. The working electrode was a CPE (geometric area 12.5 mm^2) which comprised a Teflon tube with an inner core containing a contact with a twist action. A calomel electrode and a platinum wire were used as the reference and auxiliary electrode, respectively.

A conventional flow injection analysis apparatus arrangement was used (Fig. 2). The voltammetric detector consisted of a canal thin layer cell (Bioanalytical Systems LC-4A) which was modified in the laboratory in such a way that the outlet of the mobile phase also acted as the counter electrode; the reference electrode was downstream of the working electrode and near the counter electrode.

Amperometric measurements were made with a Metrohm Model 641 VA detector and recorded on a Konik KNK-801-201 recorder. Flow injection experiments were carried out using a gas-propelled carrier stream. A six-part injector valve (Rheodyne 5060) with a 100-μl loop was used for sample injection. All connections were made with 0.50-mm i.d. Teflon tubing and the distance between valve and detector was 60 cm. The working electrode was a carbon paste electrode (geometric area 7.1 mm^2). A silver–silver chloride–saturated potassium chloride electrode was used as a reference electrode, the auxiliary electrode being a stainless steel wire.

Reagents

The MXT ampoules (Novantrone) were kindly supplied by Lederle Laboratories Division. MXT is a hydroscopic dark solid supplied as a sterile, aqueous solution containing mitoxantrone hydrochloride equivalent to 2 mg ml^{-1} of mitoxantrone free base, with sodium chloride, sodium acetate and acetic acid as inactive ingredients. The product does not contain antibacterial preservatives. Solutions of MXT were prepared daily by dilution of the sterile solution or the drug solid (84% pure) with the background electrolyte solution (0.1 M perchloric acid, pH 1.12). The samples were stored in the darkness at room temperature to avoid photochemical decomposition. The carrier solution was degassed with helium. Carbon paste electrodes were prepared by mixing 1.8 ml of paraffin oil (Uvasol, Merck, Darmstadt, Germany) with 5 g of spectroscopic grade graphite powder (Ultracarbon, Dicoex, Bilbao, Spain). All solutions were prepared using water purified by distillation and passed through an ion exchanger.

Procedure

Cyclic voltammograms were recorded in 1×10^{-6} M MXT at pH 1–12 using a fresh carbon paste surface for each experiment. A short preconcentration time at open circuit was used before each measurement. This was accomplished by immersing the CPE in a stirred MXT solution (1000 rpm) for 30 s. Then a quiescent period of 10 s (the last 5 s under electrolysis at the initial potential) was allowed before commencing the potential scan at a rate of 100 mV s^{-1}.

The activation step of the CPE surface was carried out by the following method: after each measurement the CPE was immersed in 0.1 M perchloric acid free of MXT and maintained for 30 s at a potential of −1.5 V; the CPE was then dried carefully and was prepared for the next measurement.

In FIA experiments the carrier stream was degassed with helium for 10 min and then pumped through the electrochemical cell. When a steady background had been obtained, the analyte solution was injected and the change in current recorded. The carbon paste was packed into the well of the working

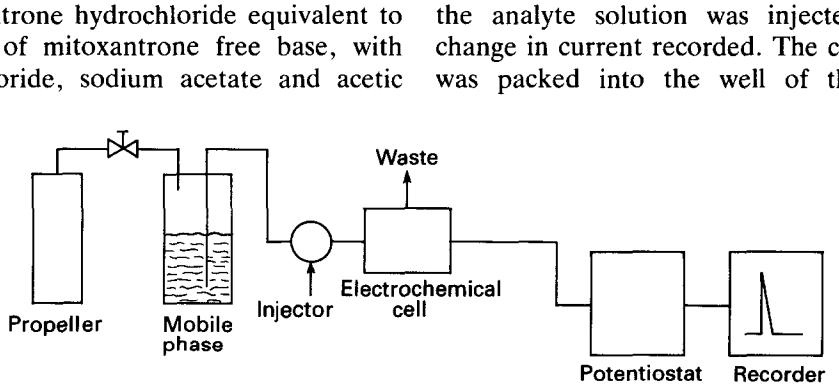

Figure 2
The FIA system.

electrode to a depth of 1 mm and the surface was smoothed on to a white paper sheet placed over a flat glass stand. The carbon was renewed daily before the start of the experiment.

Results and Discussion

Electroactivity of MXT on CPE

The electrochemical behaviour of MXT was examined at pH 1–14; two anodic peaks were observed in this pH range. The first is reversible when the cyclic scan is reversed before the second anodic peak (Fig. 3). The peak potential shifts to more negative values as the pH increases according to the equation for the first anodic process:

$$E_p\ (V) = -5.78 \times 10^{-1}\ pH + 0.795$$
$$(r = 0.997,\ n = 11;\ pH = 1\text{–}12).$$

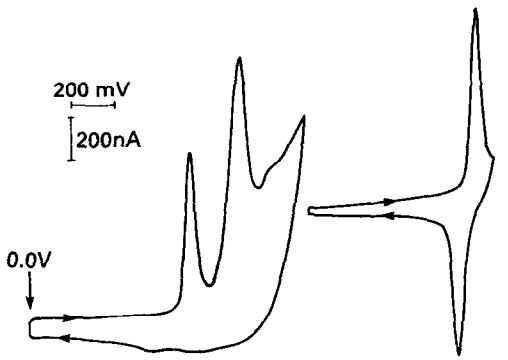

Figure 3
Cyclic voltammograms of 1.10^{-6} M MXT in 0.1 M perchloric acid.

This peak which is of more value to the analyst can be attributed to oxidation of the —OH groups to produce the quinone [9–11]. The second process shifts with a similar slope and can be attributed to the irreversible oxidation of the aromatic amino-groups [12–14]. Other reactions which break the molecule may occur on the electrode surface resulting in a loss of the reduction peak on the reverse scan.

The most sensitive signals were observed in 0.1 M perchloric acid. At this pH the difference between the anodic and cathodic peak potentials are smaller than at other pH values. Therefore, for the next voltammetric and FIA experiments 0.1 M perchloric acid was chosen. At this pH, the stability of MXT was demonstrated by successive recording of its visible spectrum which remained practically independent of time.

The responses obtained between each electrode or between each measurement with the same electrode surface were not reproducible so that an activation step was necessary before each measurement. With the MXT solution the analyte was strongly adsorbed on the working electrode at open or closed circuit. In order to activate the electrode surface the technique described under Procedure was used and a relative standard deviation of 0.98% was obtained for the voltammetric signal ($n = 10$).

When this activation step was used the effect of MXT concentration on the first peak current was shown to follow the equations:

First linear range (2.5×10^{-9}–2.10^{-8} M):

$$I\ (nA) = 5.74 \times 10^{9}\ [MXT] - 9.61$$
$$(r = 0.9996;\ n = 4)$$

The detection limit was 2.5×10^{-9} M.

Second linear range (2.10^{-8}–5.10^{-7} M):

$$I\ (nA) = 1.1 \times 10^{9}\ [MXT] + 82.73$$
$$(r = 0.9980;\ n = 4).$$

Both calibration graphs were derived for a preconcentration time of 4 min.

Owing to the laborious nature of the direct voltammetric method, an FIA method with amperometric detection based on the first anodic process was developed. Use of an FIA system avoids electrode poisoning and allows faster measurements without previous activation.

Optimization of variables in the FIA method

The first parameter optimized in the FIA metehod was the working electrode potential in the range +0.65 to +1.05 V. Figure 4 shows peak current as a function of working potential in 0.1 M perchloric acid when 1.10^{-5} M MXT was injected. It can be seen that at about the first process potential there is a relative maximum; for higher potentials, the peak current increases and exceeds the second process potential. Thus, current is independent of the working potential. Following this study + 0.90 V was chosen as the optimum potential for the working electrode.

Another important parameter was the flow rate which was adjusted according to the specific requirements of the system. Peak height and peak width depend largely on this parameter because the dispersion of the sample plug during its passage from the in-

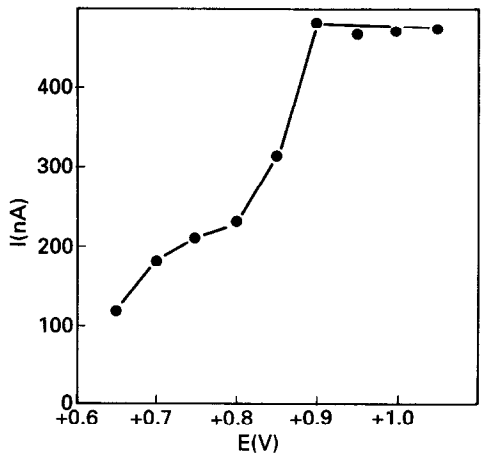

Figure 4
Peak current as a function of working electrode potential. Flow rate 2 ml min^{-1}.

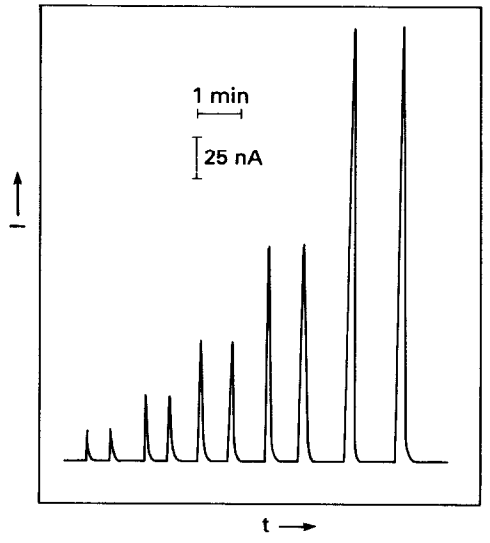

Figure 5
Influence of MXT concentration on peak currents. Concentrations were 2.5×10^{-7}, 5.10^{-7}, 1.10^{-6}, 2.10^{-6} and 4.10^{-6} M.

jection point to the detector depends on the time taken. The flow rate effect for a MXT concentration of 1.10^{-5} M and a working potential of +0.90 V was examined. Peak intensity increased linearly with the flow rate in the range studied (1–4 ml min^{-1}), and simultaneously the peak width decreased linearly. Therefore, for the next experiments the highest flow rates possible in the gas propelled system were chosen.

Using working conditions of +0.90 V and a flow rate of approximately 4 ml min^{-1} the current reached its baseline value about 25 s after injection, allowing a maximum sampling frequency in the FIA system of about 120 samples h^{-1}.

The accuracy of results was estimated by repeated injection of 1.10^{-5} M MXT using optimum conditions. The mean peak current found was 83.87 nA; the range was 82.5–85.0 nA (n = 10) and the relative standard deviation was 1.1%.

Determination of mitoxantrone

The relationship between the current peak and the amount of analyte injected (Fig. 5) produced a linear calibration plot for 5.10^{-8}–1.10^{-5} M MXT using optimum conditions. Treatment of this data by the method of least-squares gave the equation:

$$I\,(\text{nA}) = 6.27 \times 10^{7}[\text{MXT}]/M + 3.53 \quad (r = 0.998,\ n = 11)$$

The detection and the determination limits (three and 10 times the standard deviation of the background noise [15]) were about 1.1×10^{-7} and 5×10^{-7} M, respectively.

The proposed voltammetric FIA method was tested by analysis of an ampoule of sterile solution of MXT (commercial name Novantrone) containing a MXT concentration of 4.5×10^{-3} M. A 22 µl volume of the solution was diluted to 100 ml with 0.1 M perchloric acid. The concentration of MXT was then determined by a standard addition method. A mean concentration of 4.9×10^{-3} M was obtained with a relative standard deviation of 1.2% (n = 5). This also shows that the results obtained are in good agreement with those achieved using linear sweep voltammetry on a hanging mercury drop electrode (5.04×10^{-3} M, RSD = 1.37%, n = 5). The proposed FIA method has the advantage of fast sampling and is a reproducible method that can be applied to the analysis of Novantrone ampoules which do not contain other chemical compounds which are oxidizable at +0.9 V.

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