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A study of the electrochemical oxidation of Navelbine*

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Abstract: The search for drugs with cytostatic activity and with better pharmacokinetic features led to the synthesis of Navelbine® (5′-noranhydrovinblastine) which is a structural modification of antitumour *Vinca* alkaloids. The new drug Navelbine has high liposolubility, a lower toxicity and increased antitumour activity. The electrochemical oxidation of Navelbine was studied over a wide pH range (1.2–12.8) at a glassy carbon disc electrode in buffered aqueous media using differential pulse and cyclic voltammetry. The anodic oxidation mechanism is a very complex, pH dependent, multistep electron transfer process with coupled homogeneous chemical reactions.

Keywords: Vinca *alkaloids; cancer chemotherapy; electroanalysis; anodic oxidation mechanism; differential pulse voltammetry; cyclic voltammetry.*

Introduction

Antitumour *Vinca* alkaloids have been studied chemically, biologically, pharmacologically and clinically, and used in the treatment of human cancers for more than 20 years [1–2]. However, their antitumour activities are associated with toxic side effects, myelosuppression and neurotoxicity. These are either related to the alkaloid basic structure, which comprise an indole and a dihydroindole nucleus linked together, or arise from alkaloid metabolites and/or degradation products. In fact these alkaloids are representative of the 'spindle poisons', as they interfere with the polymerization of tubulin, a ubiquitous structural protein responsible for the building-up of the microtubule system and the mytotic spindle that appears during cell division. These cytotoxic compounds act by preventing the formation of the spindle, which consequently inhibits the polymerization of tubulin into microtubules. This type of drug can be evaluated by the simple and reliable test of monitoring polymerization and depolymerization of tubulin *in vitro* — this means that the drug will also be active *in vivo*.

Among the *Vinca* alkaloid type of compounds that have been synthesized, Navelbine® (5′-noranhydrovinblastine) [3–6] presents an original spectrum of biological action: lower toxicity, high liposolubility, possibility of oral administration and attack on some tumours untouched by other drugs.

Due to the severe side effects of the *Vinca* alkaloid chemotherapeutic drugs for the treatment of neoplastic diseases, it is important to develop methods of continuous monitoring of the concentration of these drugs in plasma and urine during treatment and thence be able to optimize the dosage administrated to the patients [7–9].

In this paper the results of the electrochemical oxidation behaviour of Navelbine are presented. This will contribute to a better understanding of its mechanism of action and will enable continuous electrochemical therapeutic drug monitoring of this compound in conjunction with HPLC separation.

Experimental

Chemicals and solutions

The solution of 5′-noranhydrovinblastine was prepared from the commercially available Navelbine (Pierre Fabre, Paris, France) used without any further purification. A stock solution of Navelbine with a concentration of

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1 mg cm^{-3} was prepared in 0.2 M NaCl and kept refrigerated (2–8°C).

All other reagents were AnalaR grade, the water used for the solutions was tridistilled, once over alkaline permanganate, and the experiments were carried out in buffer solutions (0.2 M) over a wide pH range (1.2–12.8, Table 1).

The experiments were carried out at room temperature (19–22°C).

Apparatus and procedures

The working electrode was a glassy carbon disc of 3.50 mm radius. The reference electrode was a saturated calomel electrode (SCE) and the auxiliary electrode a platinum foil.

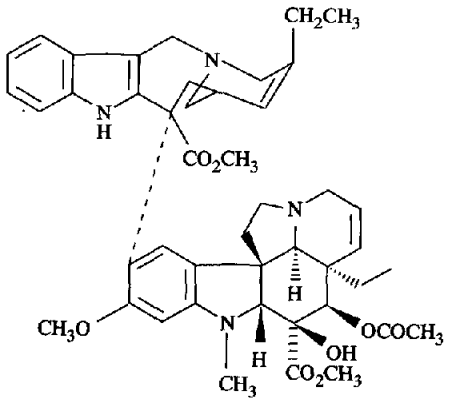
Electrode potentials were controlled and currents were measured using a Princeton Applied Research Corp. (PARC) Model 174A Polarographic Analyser with a PAR RE0074 X–Y recorder. Each solution was scanned over a potential range from 0.0 to +1.2 V vs SCE. The scan rate for differential pulse voltammetry was 5 mV s^{-1} and for cyclic voltammetry 50 mV s^{-1}.

Results and Discussion

The electrochemical oxidation of Navelbine was studied over a wide pH range (1.2–12.8) at a glassy carbon disc electrode in buffered aqueous media using differential pulse and cyclic voltammetry.

The electrochemical behaviour can be understood by considering the structure of Navelbine (Scheme 1).

This drug is different from the naturally-occurring compounds of the *Vinca* alkaloid type because it has an eight-membered ring in place of a nine-membered ring. This means that the protonation of the nitrogen in the ring



Scheme 1
The structure of Navelbine.

can be followed by the opening of this eight-membered ring.

The differential pulse voltammograms for Navelbine, at concentration 10^{-5} M, in different buffer supporting electrolytes show (Fig. 1) that the anodic oxidation mechanism is a very complex multistep electron transfer process coupled with homogeneous chemical reactions and depends on the pH. There are several electrochemical steps. For example, at pH 4.5 in 0.2 M acetate buffer, peak potentials, E_p, appear at 0.10, 0.23, 0.35, 0.70 and 0.86 V, but it is very difficult to evaluate all of them as, for the concentration used, most appear only as small shoulders so only the main peak, at E_p = 0.70 V, will be discussed. The plot of variation of peak potential, E_p, with pH (Fig. 2) shows that the peak potential changes to less positive values when the pH is increased. The dashed line in the figure corresponds to a variation of 59 mV per unit of pH and the correlation obtained with the E_p values implies that the number of electrons transferred is one and that there exists a fast deprotonation step before the electron transfer occurs. In acid media the E_p is more positive, and the peak current is

Table 1
Supporting electrolytes

Buffer (diluted to 100 ml)			pH
0.2 M KCl	(25.0 ml) + 0.2 M HCl	(42.5 ml)	1.2
0.2 M KCl	(25.0 ml) + 0.2 M HCl	(6.5 ml)	2.0
0.2 M NaOAc	(3.7 ml) + 0.2 M HOAc	(46.3 ml)	3.4
0.2 M NaOAc	(13.2 ml) + 0.2 M HOAc	(36.8 ml)	4.5
0.2 M NaOAc	(41.2 ml) + 0.2 M HOAc	(8.8 ml)	5.4
0.2 M Na_2HPO_4	(30.5 ml) + 0.2 M NaH_2PO_4	(19.5 ml)	6.9
0.2 M Na_2HPO_4	(47.4 ml) + 0.2 M NaH_2PO_4	(2.7 ml)	8.1
0.025 M $Na_2B_4O_7$	(50.0 ml) + 0.2 M NaOH	(3.0 ml)	9.3
0.2 M KCl	(25.0 ml) + 0.2 M NaOH	(6.0 ml)	12.1
0.2 M KCl	(25.0 ml) + 0.2 M NaOH	(42.0 ml)	12.8

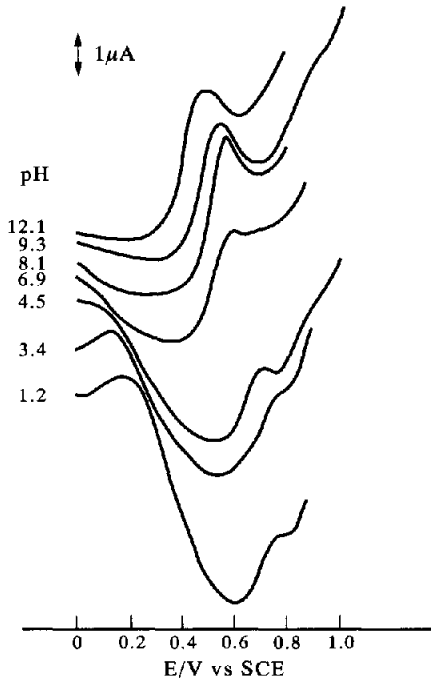


Figure 1
Differential pulse voltammograms of Navelbine, 10^{-5} M, in different buffer solutions at different pH values (see Table 1). Pulse amplitude 50 mV, scan rate 5 mV s^{-1}.

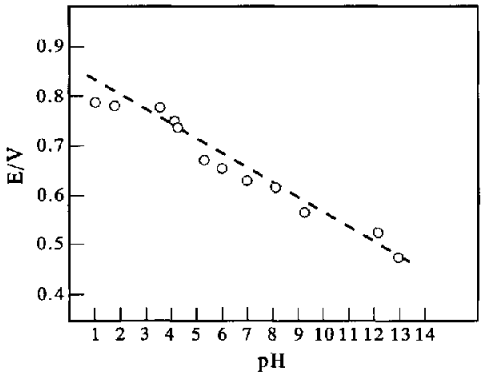


Figure 2
Variation of differential pulse voltammetry peak potentials of Navelbine with pH, in buffer solutions.

lower, so the rate constant, k_a, is smaller, i.e. it is more difficult to oxidize the compound.

Cyclic voltammetry (Fig. 3) showed that the anodic oxidation of Navelbine was an irreversible process for all pH values and a strong adsorption of the products of the electrochemical reaction on the surface of the electrode was always observed, so that a second cycle showed no peak.

Assuming that the proton and electron transfer take place separately and that proton

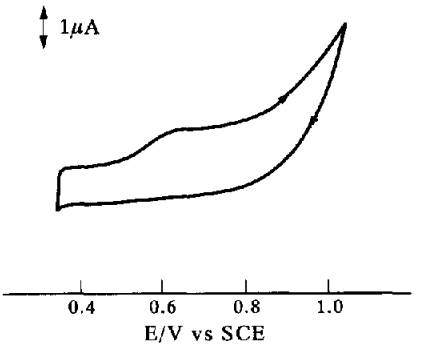


Figure 3
Cyclic voltammogram of Navelbine, 10^{-5} M, in a solution of pH 9.3 (0.025 M $Na_2B_4O_7$/0.2 M NaOH). Scan rate 50 mV s^{-1}.

transfers between oxygen and nitrogen bases in aqueous solutions are very rapid, a CE type mechanism with a pre-equilibrium proton transfer followed by a second rate determining step (rds) electron transfer can be proposed

$$HS \overset{K}{\rightleftarrows} S^- \underset{\text{rds}}{\overset{k}{\underset{-e}{\rightarrow}}} S\cdot \rightleftarrows \text{Products.} \qquad (1)$$

The product S may dimerize, polymerize, oxidize or cleave. From the results obtained it is most probable that Navelbine oxidizes and subsequently dimerizes forming an unreactive film on the surface of the electrode.

Conclusions

An understanding of the structure–activity relationship among the *Vinca* alkaloid type drugs for their effective use as cancer chemotherapeutic drugs is necessary as well as a basic knowledge of the cell constituents, of the scheme of DNA, RNA and protein synthesis and of the principles of drug action through transport, metabolism and elimination. The search for new anticancer drugs must be based on a high level of research of all the mechanisms involved using sensitive and specific methods. Anodic oxidation of Navelbine showed that the mechanism of oxidation is by electron transfer with coupled homogeneous chemical reactions. Navelbine should be able to be determined in plasma and urine using appropriate HPLC separation with electrochemical flow detection, permitting continuous electrochemical therapeutic drug monitoring.

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