Electrochemical Behavior of Vinblastine Sulfate at Platinum in Acetonitrile

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Summary: Cyclic, normal-pulse, reverse-normal-pulse, differential-pulse and square-wave voltammetry of vinblastine sulfate was investigated in acetonitrile containing 0.2 M sodium perchlorate without and with 21.4 mM pyridine present in the background solution. Vinblastine sulfate gave a single anodic peak representing irreversible electrochemical oxidation in all cases. Behavior of vinblastine sulfate was similar in the presence/absence of pyridine. Well-formed voltammograms were obtained in the absence of pyridine.

Introduction

Vinblastine is a chemotherapeutic drug that is given for the treatment of leukaemia, lymphoma, breast and lung cancer. It is in the form of colorless liquid that is injected intravenously. Its trade names are velbe (Eli Lilly) and vinblas (Pharmadec Lahore-Pakistan) [1].

Chemically, vinblastine was discovered as one of the vinca alkaloids that inhibited the polymerization of tubulin, resulting in the disappearance of the microtubular spindle and as a consequence they caused the arrest of cell division in their metaphase. Vinblastine was first isolated by Robert Noble and Charles Thomas Beer from the Madagascar periwinkle plant. Vinblastine's utility as a chemotherapeutic agent was first discovered when it was crushed into tea. Consumption of the tea led to a decreased number of white blood cells; therefore, it was hypothesized that vinblastine might be effective against cancers of the white blood cells such as lymphoma. In humans, the maximum tolerated dose of vinblastine (single agent) was approximately 0.3 $mgkg^{-1}$ [2]. There had been limited information in the literature on the metabolism of vinblastine. 4-Deacetylvinblastine had been reported to be a urinary metabolite of vinblastine in dog and human, based on thin layer chromatographic analysis. In human body half life of vinblastine had been found t $_{\frac{1}{2}}$ be 24.8 hours and its metabolism, *via* hepatic route. Vinblastine had been metabolized to deacetyl-vinblastine primarily in liver. Since 4-acetyl group of vinblastine was known to be chemically labile, deacetylvinblastine had been reported as a metabolite. This compound was found to be more biologically active on a weight basis than the parent compound both *in vitro* and *in vivo*. Its cytotoxic activity was found equal to or greater then that of parent compound. No other biologically active metabolites appeared to be present in urine or in stool. However, deacetylvinblastine was not found in human plasma. Animal studies had shown the higher sensitivity of male as compared to female. This might be due to the fact that vinblastine hardly penetrated into the muscles. Because males possessed more of this poorly penetrated compartment, at equal dose levels (in $mgkg^{-1}$ body weight) the exposure of the other tissues in males was found to be higher than in females [3].

Vinblastine was discovered to be excreted *via* biliary and renal route. Radiolabeled study had shown that little metabolic conversion occurred from vinblastine to deacetylvinblastine during first few hours after dosing. Even after twenty four hours a considerable amount of vinblastine retained in body thus its clearance was relatively slow from human body [4].

Electroanalytical techniques proved to be an effective tool for the mechanistic studies of various natural products of medicinal importance. Cyclic, normal and differential pulse voltammetry had been employed to study electrochemical behavior of the oxidation of indole alkaloids *i.e.* vinblastine, catharanthine, and vindoline in mixed aqueous/ organic media. Differential pulse voltammetry at

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carbon paste anodes in pH 5.6 acetate buffer in ethanol/water (1:1) was found to be more efficient as compared to other techniques. The detection limit for vinblastine was found to be lowest in contrast to catharanthine and vindoline and evidence was provided by means of calibration graphs. Results obtained by cyclic voltammetry as well as by pulse techniques suggested that electron transfer was preceded by deprotonation and followed by additional chemical reactions. Product of the electrode processes were reported to form mechanically unstable films on the electrode surface [5]. Under similar experimental conditions *i.e.* differential pulse voltammetry on highly polished glassy carbon electrode in ethanol/water (1:1) at pH = 5.6 was also employed to the mixtures of catharanthine, vinblastine and vindoline had provided a method for quantitative determination of a mixture of catharanthine and vinblastine or catharanthine and vindoline at low concentrations [6].

The anodic oxidation mechanism of the vinca alkaloids including vinblastine and all with closely similar structures was studied at a glassy carbon disk electrode in buffered aqueous media using differential pulse and cyclic voltammetry. The effect of pH on the mechanism showed that it was a complex multistep electron transfer with deprotonation steps involved and that the final products, dimers or polymers, adsorbed strongly to the electrode surface, forming an unreactive film on electrode surface. The electrochemical differential pulse voltammograms showed a correlation between the vinblastine derivatives. These effects may be due to the metabolite and/or degradation products of the compounds and resulted from how they were attached to, or transported across, cellular structures such as cell membranes [7].

Dimerization of vindoline, at controlled potential at platinum electrode resulted in the formation of 10,10'-*bis*vindoline in 60% yield had also been reported [8].

Coupling reactions of catharanthine and vindoline *i.e.* with ferric ion [9], and borane complexes [10] had been reported.

It has been revealed that a regio- and stereoselective didehydrodimerization procedure, in which key step involved the anodic oxidation, resulting in the conversion of α-anilinoacrylic alkaloids belonging to the Aspidosperma class, typified by tabersonine and its 3-oxo derivative, into the hitherto unknown 16, 10'-didehydro dimers [11].

Studies designed for the preparation of vinblastine and its analogs had also been reported [12].

It has been found that various aspects of vinblastine and its reactants had been studied previously by the use of various electroanalytical methods [13]. Analysis of these alkaloids by electrochemical methods had been summarised [14] and reviewed [15].

In order to investigate the mechanistic studies involved in the voltammetric oxidation of vinblastine sulfate, cyclic and pulsed voltammetric techniques were carried out at the platinum semi-micro electrode, in 9:1 acetonitrile-water mixture containing 0.2 M sodium perchlorate. Results acquired clearly indicated the occurrence of electron transfer followed by subsequent reactions [16].

The interaction of vindoline and catharanthine within reaction media was studied providing a detailed account for the complicated electrochemical processes [17]. The electrochemical oxidation of catharanthine in the presence of vindoline was performed in MeCN-Et_4NClO_4 at controlled potential yielded (16'*S*)- and (16'*R*)-anhydrovinblastine (52 % and 12%, respectively) [18].

Electrochemical methods coupled with other analytical technique such as reversed-phase high-performance liquid chromatography was also being reported as a detection tool for vinblastine and related drugs in biological samples [19]. Electroanalytical techniques were also found to play an important role in structural elucidation of newly modified analogs of vinca alkaloids [20].

Differential pulse polarographic methods for the determination of the antineoplastic agents, vincristine and vinblastine at ng/mL, in biological fluids such as plasma and urine had also been reported. The most important point in the electroanalysis of these molecules was found to be their adsorption on the electrode surface. The peak potentials and limiting currents were discovered to be pH dependent [21].

Cyclic voltammetry had been used to investigate the electrochemical behavior of the anti-cancer herbal drug emodin at glassy carbon electrode. Using the established method without pretreatment and pre-separation, emodin in herbal drug was determined with satisfactory results. Moreover, the electrode process dynamics parameters were also investigated by electrochemical techniques [22]. Various anti-cancer drugs had been investigated by means of electroanalytical methodology to interpret their redox behavior [23-26].

The use of electrochemical methods to obtain relevant information about drugs, mechanism of action and analysis of cellular events had been found to be quite informative. Electrochemical studies could furnish an enormous amount of evidence regarding the mechanisms of biological electron transfer processes [27]. The goal of current studies was to use electroanalytical methods to characterize the oxidation of vinblastine sulfate in acetonitrile; electrochemical oxidation reaction properties could contribute to understanding the biological applications mentioned above [28].

Result and Discussion

Voltammetry of 0.22 mM Vinblastine Sulfate at Platinum Electrode in Pure Acetonitrile Containing 0.2 M Sodium Perchlorate

Tables-1 and 2 represented the dependence of anodic peak potential on concentration and scan rate. As peak potentials were shifted to more positive values when the scan rate was increased, this was presumably consistent with the occurrence of chemical reactions following a reversible electron transfer process.

Table-1: Electro-oxidation of vinblastine sulfate on platinum. Dependence of anodic peak potential on concentration of vinblastine sulfate.

Scan rate	$\partial E_p/\partial \log C$	$\partial E_{p/2}/\partial \log C$
Vs^{-1}	mV dec^{-1}	mV dec^{-1}
0.1	126.6	105
0.03	100.7	97.0

Table-2: Electro-oxidation of vinblastine sulfate on platinum. Dependence of anodic peak potential on scan rate.

Vinblastine sulfate	$\partial E_p/\partial \log v$	$\partial E_{p/2}/\partial \log v$
mM	mV dec^{-1}	mV dec^{-1}
0.033	21.2	5.3

Fig. 1 elucidated a typical normal pulse voltammogram for the oxidation of 0.22 mM vinblastine sulfate in acetonitrile containing 0.2 M sodium perchlorate, at platinum electrode. It exhibited a single irreversible oxidation wave at about 1.3 V. The variation of peak current with pulse width for normal pulse voltammetry of 0.22 mM vinblastine sulfate was found to be linear upto 0.07 s and followed by a decreasing trend with increasing pulse width up to 0.10 s. Such behavior might be attributed to the passivation of the electrode surface or to the inactivation of the electroactive species by some follow up chemical reactions.

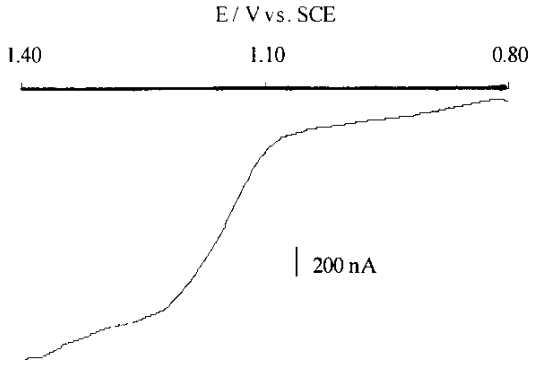

Fig. 1: Normal pulse voltammogram of 0.22 mM vinblastine sulfate at platinum electrode.

Fig. 2 represented reverse normal pulse voltammogram for the oxidation of 0.22 mM vinblastine sulfate in acetonitrile containing 0.2 M sodium perchlorate at platinum electrode, illustrating an irreversible peak. Variation of peak current with pulse width for reverse normal pulse voltammetry showed a gradual increase in current with increasing pulse width. This response was found to be opposite to that of normal pulse voltammetry showing the

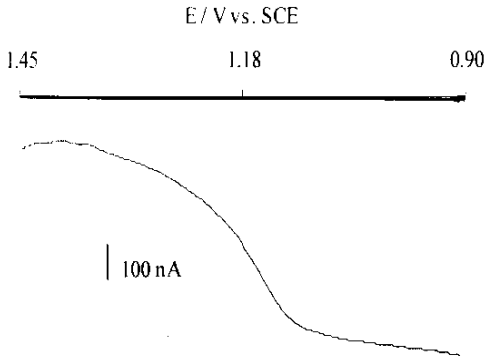

Fig. 2: Reverse normal pulse voltammogram of 0.22 mM vinblastine sulfate at platinum electrode in acetonitrile containing 0.2 M sodium perchlorate.

characterization of the products during the course of reaction. As in normal pulse voltammetry, the usual practice was to select a base potential in a region where the electroactive species of the interest did not react at the electrode. On the contrary, reverse pulse voltammetry was found to characterize the reaction products. In case of voltammetric oxidation of 0.22 mM vinblastine sulfate, the voltammetric response in case of both techniques (normal pulse voltammetry and reverse normal pulse voltammetry) was found to be similar *i.e.* a single oxidation wave representing one electron transfer which was suggested to be taken out from the indole part of the catharanthine moiety of vinblastine.

A square wave voltammogram of 0.22 mM vinblastine sulfate was recorded showing irreversible electrode process. The relationship of square root of frequency with peak current was established as per Fig. 3, which clearly indicated the occurrence of diffusion controlled process. Variation of summit potential and summit current with pulse height for differential pulse voltammetry was investigated as shown in Fig. 4.

Scan Rate Dependence for Cyclic Voltammetry of 0.033 mM Vinblastine Sulfate

Cyclic voltammetry of 0.033 mM vinblastine sulfate was carried out in pure acetonitrile at different scan rates to illustrate the mechanistic pathways adopted by the electroactive species. It was evident that the current of these voltammograms

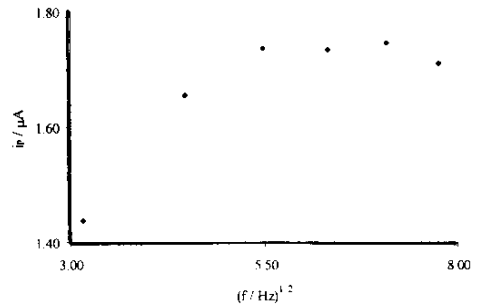

Fig. 3: Variation of square-wave-voltammetric-peak current with square root of frequency. Concentration of vinblastine sulfate 0.22 mM.

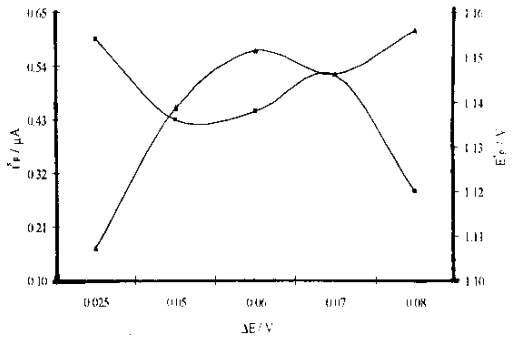

Fig. 4: Variation of summit potential, and summit current with pulse height in differential pulse voltammetry of 0.22 mM vinblastine sulfate.

increases with increasing scan rate although the peak potential remained the same. A single peak appeared at the potential near to 1.12 V which corresponded to the electron transfer from catharanthine moiety of the parent compound. Variation of peak current with square root of scan rate (Fig. 5) indicated the occurrence of diffusion controlled processes.

The variation of peak potential with square root of scan rate showed complex behavior *i.e.* the peak potential inclined to decrease followed up by gradual increase. Relationship of peak current with log of scan rate indicated gradual increase of peak current with increasing scan rate. These results obtained from cyclic voltammetry of vinblastine sulfate clearly suggested that it was an irreversible and diffusion controlled process, evidence was being

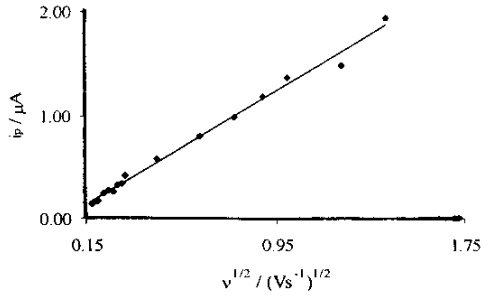

Fig. 5: Variation of peak current with square root of scan rate for cyclic voltammetry of 0.033 mM vinblastine sulfate.

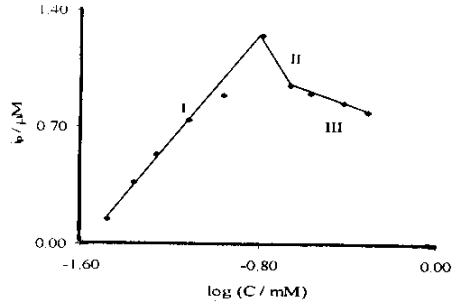

Fig. 6: Variation of cyclic-voltammetric-peak-current with log concentration of vinblastine sulfate. Scan rate 0.03 Vs^{-1}.

provided by the relationships of scan rate with peak current and peak potential.

Concentration Dependence of Vinblastine Sulfate at Low and High Scan Rate:

Comparative studies were carried out by using cyclic voltammetry at higher and lower scan rates, respectively in order to evaluate different physical parameters for the electrochemical oxidation of vinblastine sulfate. Graphs were plotted to describe the dependence of peak current on log of concentration at low scan rate as per Fig. 6. The peak current was found to increase with increasing concentration followed by a rapid decrease, both at low and high scan rates. It was suggested that the electrode surface was being covered by adsorbed products of the reaction affecting the overall efficiency of the electrode surface with increasing concentration of the analyte.

Voltammetry of Vinblastine Sulfate in the Presence of Pyridine

Voltammetry of vinblastine sulfate was carried out in acetonitrile containing 0.2 M sodium perchlorate. The studies were done in the presence of 21.4 mM pyridine in the above background solution. The use of pyridine as a solvent was suggested owing to its certain characteristics, like being polar and aprotic, so it did not interfere with the actual redox process. Furthermore, being an important precursor for various pharmaceuticals and a part of the natural biological system in the form of various compounds such as nicotinamide dinucleotide, it was suggested to observe the behavior of the vinblastine to mimic its redox behavior within physiological system.

Vinblastine sulfate gave a single anodic peak representing irreversible electrochemical oxidation in the presence of pyridine as shown in Fig. 7. The relationship between peak current and potential versus square root of scan rate was found to be linear thereby depicting a diffusion controlled process.

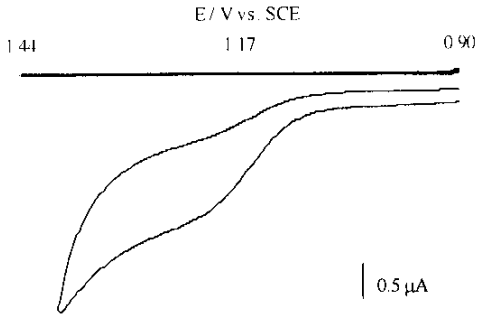

Fig. 7: Cyclic voltammogram of 0.11 mM vinblastine sulfate in acetonitrile containing 0.2 M sodium perchlorate, and 21.4 mM pyridine.

Differential pulse voltammetric behavior of vinblastine was investigated in the presence of pyridine. Results acquired were similar to those of cyclic voltammetry as shown in Fig. 8. Dependence of summit potential on peak width was studied and that was found to be linear in two regions with decreasing trend. Normal pulse voltammetry of 0.11 mM of vinblastine sulfate in the presence of pyridine was carried out. Influence of peak width on peak current was investigated and found to show gradual rise in peak current with increasing peak width as shown in Fig. 9. Investigation of the behavior of

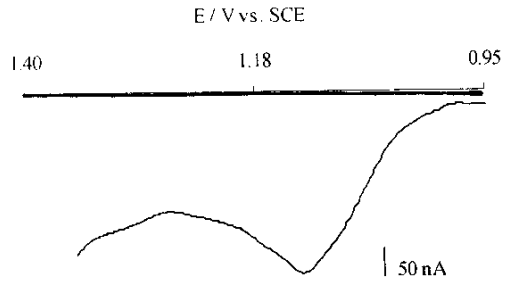

Fig. 8: Differential pulse voltammogram of 0.11 mM vinblastine in acetonitrile containing 0.2 M sodium perchlorate, and 21.4 mM pyridine.

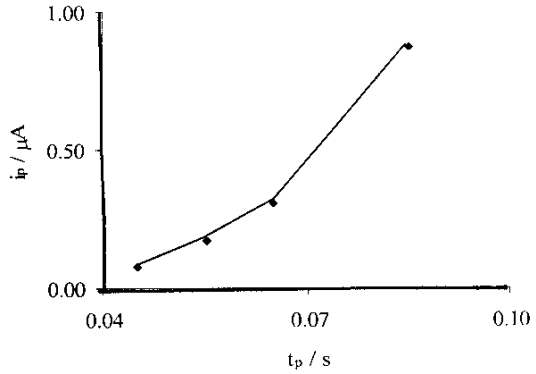

Fig. 9: Variation of normal-pulse-voltammetric limiting-current (i_p) with pulse width for 0.11 mM vinblastine sulfate + 21.4 mM pyridine at platinum electrode in acetonitrile containing 0.2 M sodium perchlorate.

vinblastine sulfate in the presence of pyridine confirmed the irreversible single electron transfer process as revealed by appearance of an oxidation wave in all techniques that have been employed. The voltammetric behavior of vinblastine sulfate was found to be comparable in the presence or absence of pyridine. However, in the absence of pyridine, shifts in the anodic peak potential were more pronounced. Future studies would emphasize the electrochemical determination of the precursors of vinblastine sulfate *i.e.* vindoline and catharanthine and their analogs. Much stress would be laid on the coupling of these derivatives *via* electrochemical methods followed by the study of the redox behavior of resulting products.

Experimental

Chemicals and Materials

Specification of chemicals and materials used were vinblastine sulfate (Eli Lilly France S. A.), vinblas (Pharmedic Laboratories Pvt. Ltd.), acetonitrile (E-Merck Germany), sodium perchlorate (E-Merck Germany), pyridine (E-Merck Germany) and γ-alumina (0.05micron) mesoporous (moleculer sieve alumina) of EG&G. Acetonitrile was double distilled and sodium perchlorate was recrystallized to perform the experiments.

Polishing of Working Electrodes

Polishing was done by dipping the working electrode in 5% nitric acid for five minutes prior to each scan. It was thoroughly rinsed with distilled water before introducing into the cell.

Instrumentation

Voltammetry was carried out using EG&G, Princeton Applied Research Corp, VersaStat II potentiostat. All experiments were performed in a three electrode cell containing platinum ($8x10^{-4}$ cm^2) working electrode, a platinum wire as counter electrode and saturated calomel reference electrode.

The cyclic and pulse voltammetric techniques were carried out for different concentrations of vinblastine sulfate in three-electrode voltammetry cell (working, counter and reference electrode) in following background systems:

(i) 0.2 M sodium perchlorate (recrystallized) in pure acetonitrile solution.
(ii) 0.2 M sodium perchlorate (recrystallized) and 21.4 mM pyridine in pure acetonitrile solution.

Working electrodes were polished prior to each scan. The electrodes were thoroughly washed with distilled water and dried then they were introduced in to the cell. Data were acquired using M270 electrochemistry research software on a dedicated PII microprocessor coupled to the potentiostat. All experiments were carried out at room temperature.

Conclusion

The voltammetric behavior of vinblastine sulfate was comparable in the presence or absence of pyridine. However, in the absence of pyridine shifts in the anodic peak potential were more pronounced. Peak potentials were shifted to more positive values when the scan rate was increased as shown in Tables-1 and 2. Such behavior was consistent with the occurrence of chemical reactions following a reversible electron transfer step. The presence of pyridine did not change the voltammetric responses of vinblastine sulfate except for a slight change of the residual currents.

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