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Electroanalytical Investigation of Cancer Chemotherapy Drug Vinorelbine on Disposable Pencil Graphite Electrode in Surfactant Media by Voltammetric Method*

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ABSTRACT

Vinca alkaloids have been used in the treatment of human cancers for over 50 years. In this study, a new application of disposable pencil graphite electrode is presented for the determination of Vinorelbine, which is one of the semisynthetic derivatives of *Vinca* alkaloids.

The electrochemical properties of Vinorelbine were investigated using cyclic voltammetry and adsorptive stripping voltammetry in aqueous solutions over the pH range of 2.0-12.0 in the absence and presence of anionic and cationic surfactants. Employing square-wave stripping mode (after 120 s accumulation at +0.0 V) in Britton-Robinson buffer pH 10.0 containing 3×10^{-3} M cationic surfactant, TBAB (tetra-*n*-butylammonium bromide), there was an excellent correlation between oxidation peak current at +0.75 V (vs. Ag/AgCl) and Vinorelbine concentration in range of 2.3×10^{-8} – 5.8×10^{-6} M. The limit of detection was found to be 7.5×10^{-9} M (as base form, 5.8 ng mL^{-1}). The applicability of the developed technique was tested in pharmaceutical formulations and spiked human urine samples.

1. INTRODUCTION

One of the biggest global public health issues, cancer has a negative impact on people's health and quality of life. Therefore, it is undeniable that the interest in drugs for cancer treatment has increased. On the other hand, in addition to patient safety during, the administration of drugs used in cancer treatment due to their possible toxic properties, it is crucial to maintain a safe working environment and to safeguard the health professionals who provide the treatment from the possibility of coming into touch with this class of medications. At the same time, it is extremely important to develop new methods for the determination of antineoplastic drugs in different environments.

Although some of the *Vinca* alkaloids in the herbal antineoplastic drugs class have been marketed for more than 50 years for use in cancer treatment, little is known about their mechanism of action and metabolism. Vinorelbine is a semi-synthetic derivative of Vinblastine, one of the *Vinca* alkaloids, also known as Anhydrovinblastine (see Figure 1 for its chemical structure).

The molecule was discovered by Pierre Potier and his research group (France) in 1980, and was developed in 1989 under the trade name Navelbin® IV (Vinorelbine ditartrate) for the treatment of bronchial cancer. In 1991, it was approved for the treatment of non-small-cell lung cancer. Vinorelbine is currently used in the treatment of different cancer diseases in combination with other chemotherapeutic drugs. It has been shown to be especially effective in the treatment of advanced breast cancer and non-small-cell lung cancer **[1-3].**

According to the literature review, it has been seen that liquid chromatography (LC) has been the most widely used method in the last two decades for the determination of *Vinca* alkaloids in general, Vinorelbine in particular, from different media (pharmaceutical forms, environmental samples and biological samples such as urine, blood, tissue). Mass spectrometry (MS) is preferred as a detection system especially for pharmacokinetic studies **[4-8]**. Although LC has high

sensitivity, selectivity and accuracy, it requires experienced personnel, long and laborious pre-separation processes, organic solvents, high cost devices (especially in the detection unit).

On the other hand, despite the redox active groups in the molecular structure of *Vinca* alkaloids, very few studies have been reported on their electrochemical properties due to their very complex structure [9-15]. Considering that voltammetry, which is one of the electroanalytical methods, is simple, fast, low cost, low sensitive to matrix effect and suitable for on-site analysis; it is clear that this method will be an alternative candidate for the determination of these antineoplastic drugs in the near future [16-18].

According to our best knowledge, there is only one study on the voltammetric characteristics of Vinorelbine [19]. In that study performed in 1993, the electrochemical oxidation of this molecule was investigated using cyclic and differential pulse voltammetric techniques at the surface of glassy carbon (GC) electrode in a wide pH range (1.2-12.8). Its results were revealed that the anodic oxidation mechanism of Vinorelbine is very complex and pH-dependent.

In the study presented here, the redox properties of Vinorelbine will be evaluated using commercially available graphite pencil leads as electrode material. Beginning from the end of the 1990s, pencil graphite (PG) electrodes has become an alternative to other solid electrodes due to their several outstanding properties, such as disposability, good mechanical stability, high electrochemical reactivity, low technology, low cost, and ease of preparation. Because PG electrodes are single-use, cleaning procedures are not required. Commercial mechanical pencil leads of different hardness and diameter (as a mixture of natural graphite and clay) are used in the design of PG electrodes [20-25].

Apart from the electrode material, one of the simple and inexpensive ways to increase the sensitivity and selectivity of the voltammetric method is to use surfactants at their submicelle concentrations in the electrochemical cell. [26]. Our research group performed the determination of many hydrophilic or hydrophobic compounds in surfactant-containing solutions using different carbon-based electrodes by voltammetric method. [27-31].

In view of the only one data on the electrochemical investigation of Vinorelbine, the aim of this study is to investigate the electrochemical oxidation of this compound using PG electrode in the presence of both anionic and cationic surfactants. Next, the applicability of the voltammetric approach will be tested by the analysis of pharmaceutical formulations and spiked human urine samples.

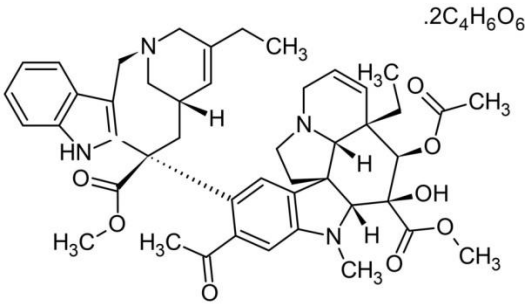

Figure 1. Molecular structure of Vinorelbine ditartrate

2. EXPERİMENTAL

2.1. Apparatus

The voltammetric measurements were performed using a Autolab PGSTAT 128N potentiostat/galvanostat (EcoChemie, the Netherlands) driven by Nova 1.1 software, and BAS C3 solid electrode cell stand unit (Bioanalytical System, BAS, USA). All experiments were conducted in a 10-mL one-compartment electrochemical cell consisting of a PG working electrode, an Ag/AgCl (3M NaCl) reference electrode (BAS, MF-2052, USA), and platinum wire auxiliary electrode (BAS, MF-1032, USA). The pH values of solutions were measured using the pH meter (WTW inoLab720) equipped with the glass-reference electrode.

For the preparation of PG electrode, mechanical pencil (Model Rotring T 0.5, Germany) as a holder and soft pencil leads (20% of clay + 74% of graphite + 6% of polymeric wax as a binder) with 6.0 cm of length and 0.5 mm diameter (Model Tombow 0.5/2B, Japan) were purchased from a bookstore. A similar way to that used in previous work [21] was applied for the electrode design. For electrical contact, the metallic head of the pencil body and the outer metallic handle were attached with copper wire. Before each measurement, 12 mm of pencil lead (total) was dipped into the solution, and then PG electrode was subjected to an electrochemical pretreatment. For this purpose, a potential of +1.4 V was applied for 60 s in selected supporting electrolyte without stirring (in the case of CV studies, at +1.8 V for 60 s). Each voltammetric recording was carried out using a new pencil lead. Following the way in our previous work [31], the effective surface area of the designed PG electrode was calculated to be 0.159 cm^2.

2.2. Chemicals and solutions

The analytical standard of Vinorelbine (as ditartrate salt, purity ≥90%) was purchased from Sigma-Aldrich. As the solubility of this compound is limited in water, dimethylsulfoxide (DMSO) was used in the preparation of its stock solution (1×10^{-3} M). The prepared solutions were stored in a refrigerator at +4 ºC, away from light when not in use. The less concentrated solutions of this compound used in calibration studies and sample analysis were prepared from the stock solutions by dilution of appropriate volume with supporting electrolyte. Analytical-grade reagents and ultrapure water (resistivity ≥ 18.2 MΩ cm) supplied from a Millipore Milli-Q purification system (USA) were used for the preparation of supporting electrolytes namely Britton-Robinson (BR) buffer (each constituent having a final concentration of 0.04 M, pH 2.0-12.0), phosphate buffer (0.1 M, pH 3.0 and 7.4) and acetate buffer (0.1 M, pH 4.8). The surfactants tested were cationic type, tetra-*n*-butylammonium bromide, TBAB (99%, Merck) and anionic type, sodium dodecylsulfate, SDS (90%, Merck). Their stock solutions (0.1 M) were prepared in water.

2.3. Measurement procedures

In preliminary studies on the electrochemical behavior of Vinorelbine, the cyclic voltammetry (CV) was applied on the surface of PG electrode. Then, the performance characteristics of the square-wave adsorptive stripping voltammetry (SW-AdSV) were determined at different pH values in the absence and presence of surfactants. Later, this technique was applied to the real samples.

The general procedure for the stripping voltammetric determination of Vinorelbine can be summarized as follows: The three-electrode system was immersed in a selected supporting electrolyte (at a desired pH) containing a selected concentration of Vinorelbine. A chosen accumulation potential (optimal, 0.0 V) was then applied to the surface of PG electrode for a suitable accumulation period of time (optimal, 120 s) while the solution was stirred at 750 rpm. Then stirrer was stopped and the solution was allowed to settle for 10 s. Afterwards, the voltammograms were recorded in a certain potential scanning range (from 0.0 V to +1.4 V) in the positive direction using the SW waveform.

2.4. Sample Preparation

For the application of the method to the pharmaceutical dosage forms, Navelbine® injectable solutions (50 mg 5 mL^{-1} Vinorelbine) were purchased from the local pharmacies. Each vial contains 69.25 mg of Vinorelbine ditartrate equivalent to 50 mg of Vinorelbine. The content of two ampoules of this formulation was mixed thoroughly. The required volume was directly transferred to the electrochemical cell containing 10 mL of Britton-Robinson buffer (pH 10.0) containing 3×10^{-3} M TBAB. Quantifications were performed using the calibration curve developed for the pure electrolyte, from the related regression equation.

Urine sample was collected in plastic container from a healthy non-smoker and drug-free volunteer on an empty stomach and just before the experiments. The sample was centrifuged at 5000 rpm for 10 minutes to remove unknown endogenous chemicals. 50 µL of urine from the clear upper part was pipetted into a 10 mL-glass tube and then spiked with 10 µL of 2×10^{-4} M Vinorelbine (model human urine sample). This sample was then completed to the volume with BR buffer (pH 10.0) unless otherwise stated. This final mixture was transferred into the electrochemical cell and subjected to SW-AdSV measurements. The determination of Vinorelbine in spiked urine sample was carried out using standard addition method with respective volumes of 45, 60, 70, 80 and 90 µL of Vinorelbine solution used above, and stripping voltammograms were recorded after each addition. The Vinorelbine-free part of the urine sample was used as a blank.

Each experiment of pharmaceuticals and urine samples was repeated three times.

3. RESULTS AND DISCUSSION

In order to understand the electrochemical response of Vinorelbine on the surface of PG electrode, the experiments were carried out using CV and SW-AdSV techniques in aqueous or surfactant-containing aqueous solutions (Note: Since the DMSO concentration is kept at ~1% (v/v) and below in the recording of CV and AdSV curves, the solutions used are expressed as "aqueous solution").

3.1. Cyclic voltammetry in aqueous solutions

At the beginning of the study, two consecutive CV curves (CVs) of 2.3×10^{-5} M Vinorelbine in BR buffer at pH 10 (the most suitable medium for analytical purposes, as will be shown later) were recorded within the potential window from 0.0 V to +1.4 V at scan rate of 100 mVs^{-1} (Figure 2). As can be seen from the first positive-going scan, the molecule was oxidized in two distinctly separated steps at about +0.73 V (Ia) and +1.00 V (IIa) with their peak currents of 11.18 µA (Ia) and 10.68 µA (IIa). When the potential scanning was continued in the cathodic region after the oxidation of the molecule, no reduction steps were observed in the studied potential range. It suggests the totally irreversible electrode reaction of Vinorelbine. On the second forward scan, the signal of the Ia was greatly reduced and the IIa step was disappeared. This formation is an important indication that the oxidation products of Vinorelbine are strongly adsorbed on the electrode surface. A similar observation was also reported previously for the electrochemical properties of Vinorelbine using GC electrode [19].

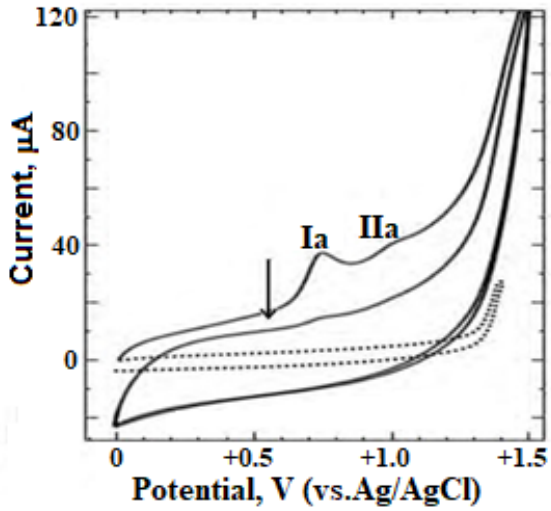

Figure 2. The repetitive CVs of 2.3×10^{-5} M Vinorelbine in BR buffer (pH 10.0). Electrode, PG; potential scan rate, 100 mVs^{-1}. Dashed lines, supporting electrolyte. Arrow indicates order of the recorded scans

In order to examine the effect of potential scan rate on the electrochemical oxidation of Vinorelbine molecule, CVs of 4.6×10^{-5} M Vinorelbine solution were recorded by increasing this parameter from 50 to 700 mVs^{-1} using the above conditions (Figure 3). As the scan rate gradually increased, an increase in the peak currents of both Ia and IIa, as well as a shift in peak potentials to slightly more positive values were observed. This supports the above-mentioned irreversibility of the electrode reaction [32].

As seen clearly from the figure, the first oxidation step (Ia) became sharper and measurable, so this step was chosen as the analytical signal in the continuation of the study.

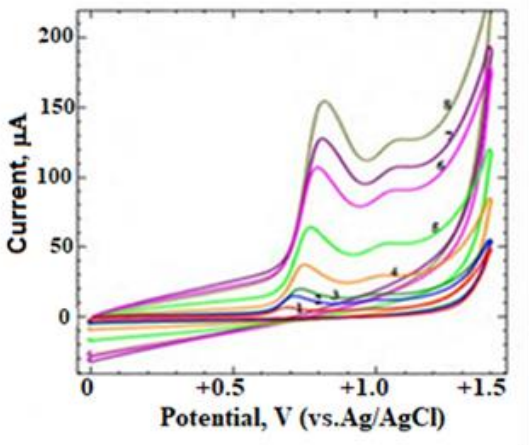

Figure 3. The CVs of 4.6×10^{-5} M Vinorelbine recorded at various scan rates: (1) 50, (2) 100, (3) 200, (4) 300, (5) 400, (6) 500, (7) 600, (8) 700 mVs^{-1}. Electrode, PG; supporting electrolyte, BR buffer (pH 10.0).

Plotting the oxidation peak current (i_p) vs. scan rate (v) gave up a straight line, which demonstrates an adsorption behavior of Ia in this medium. The equation is expressed below:

i_p (μA) = 0.12 v (mV s^{-1}) – 5.28 (r = 0.998)

On the other hand, it was also observed a linear relationship between i_p and $v^{1/2}$ according the following equation, which suggests a diffusional behavior:

i_p (μA) = 4.11 $\sqrt{v}$ (mV s^{-1}) – 34.22 (r = 0.977)

However, as can be seen from the r value of equation, the linearity in this relationship is not very good between 50 and 700 mV s^{-1}, and deviations are observed at low (50 and 100 mV s^{-1}) and high (600 and 700 mV s^{-1}) scan rate values. This case shows that the diffusion mechanism is also involved in the electrochemical reaction of Vinorelbine, but it is effective at moderate scan rates.

In order to understand the oxidation of Vinorelbine on the PG electrode, the data through a plot of log i_p vs. log v were also analyzed, according to the following equations:

log i_p (μA) = 1.30 log v (mV s^{-1}) – 1.74 (r = 0.996)

The slope value in the related equation is greater than 1.0, which proves that the oxidation process is controlled by adsorption [33]. In the light of these findings, the electrochemical behavior of Vinorelbine on the PG electrode is mainly controlled by adsorption. On the other hand, it can be said that diffusion also contributes using the scan rates between 200-500 mVs^{-1}.

Meanwhile, the relationship between E_p and log v can be expressed as follows in the range of potential scan rate studied:

E_p (V) = 0.072 log ν (mV s^{-1}) + 0.80 (r = 0.982)

For an irreversible electrode process [32], the relationship between E_p and v is defined as:

$E_p = E^0 + (2.303RT / \alpha nF) \log (RTk^0 / \alpha nF) + (2.303RT / \alpha nF) \log v$

In the equation, α is the charge transfer coefficient and n is the number of electrons transferred in the redox reaction. R (8.314 J K^{-1} mol^{-1}), T (298 K) and F (96480 C mol^{-1}) are known constants. The slope value in the E_p / log v relationship given above is 0.072. Using the related equation, the αn value is calculated as 0.81. For most completely irreversible electrode systems, the α value can be accepted as 0.5. Thus, the value n = 1.64 (≈ 2) is obtained. This value shows that two electrons per molecule are transferred for the irreversible oxidation process of the Vinorelbine molecule at the surface of PG electrode. The result obtained is consistent with the SW-AdSV finding performed in BR buffer using a modified carbon paste electrode on Vincamin, one of the indole alkaloids of the *Vinca minor* plant [13].

3.2. Stripping voltammetry in aqueous and aqueous-surfactant solutions

In the light of the above findings, in this part of the study, the electrochemical oxidation of Vinorelbine molecule was investigated in surfactant-free or surfactant-containing aqueous solutions.

3.2.1. Effect of solution pH and surfactant

At first, this part of the study was focused to examine the effect pH of the supporting electrolyte in order to understand the optimum electrochemical responses of Vinorelbine molecule. For this, using the SW-AdSV technique (accumulation potential, 0.0 V and accumulation period of time, 60s) the voltammograms of 2.3×10^{-5} M Vinorelbin were recorded in BR buffer at the range of pH 2.0-12.0 in the absence and presence of surfactants (Figure 4).

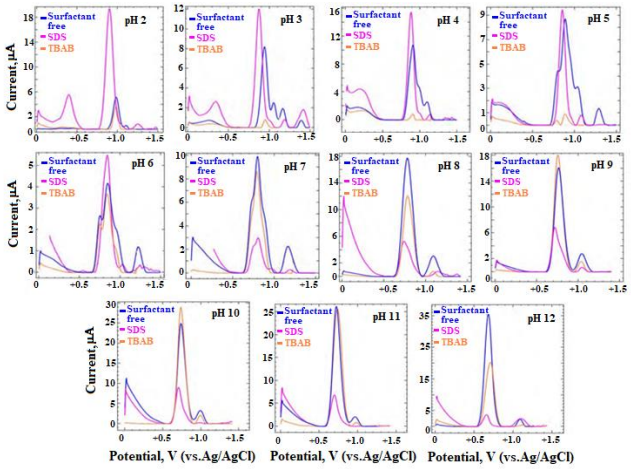

Figure 4. SW-AdSV curves of 2.3×10^{-5} M Vinorelbine in BR buffer (pH 2.0-12.0) in the absence and presence of anionic (SDS) and cationic (TBAB) surfactants. Electrode, PG; accumulation potential 0.0 V; accumulation period of time, 60 s; surfactant concentration, 5.0×10^{-3} M; SW variables: step potential, 5 mV; frequency, 25 Hz; pulse amplitude, 20 mV.

As can be seen from the voltammograms obtained in surfactant-free BR buffer solutions (shown as blue line), the anodic oxidation mechanism of the Vinorelbine molecule is pH dependent and probably involves many complex electron transfer processes. Especially in the pH range of 3.0-7.0, a large number of shoulder-shaped oxidation steps appeared and it was very difficult to evaluate these steps. Findings obtained in repeated experiments in acetate (pH 4.8) and phosphate (pH 3.0 and 7.4) buffers were in agreement with the findings of BR buffer corresponding to the same pH values (data not shown). In strongly acidic solutions (pH 2.0) and solutions above pH 7.0, these steps were separated from each other and their significance increased. Brett et al. [19] also obtained similar findings for Vinorelbine in the study performed on GC electrode.

The peak potentials shifted to the lower positive values as the pH values increased. This observation is proof that proton transfer is also involved in the oxidation process of Vinorelbine. When the relationship between the peak potential (E_p) and pH was examined, two linear regions with different slope values were obtained in the wide pH range studied:

E_p (mV) = -31.2 pH + 1037 (r = 0.992) (pH 2.0 to 6.0)

E_p (mV) = -19.0 pH + 930 (r = 0.999) (pH 7.0 to 11.0)

These findings were also consistent with those of obtained for Vincamin [13]. The reason why the correlation coefficient (r) value of the relationship in the pH 2.0 – 6.0 range is not very good is due to the possible reading error of the fundamental oxidation peak intensity due to the peak splits in the relevant range.

Due to the complexity of molecular structures of *Vinca* alkaloids, their detailed oxidation pathways were not well documented so far. Considering the electrochemical behavior of indoline-alkaloids with structural similarity [9,12], it can be assumed that the oxidation of Vinorelbine molecule involves multi-step electron transfer with the loss of protons. Subsequently, chemical reaction products (dimers or polymers) are strongly adsorbed on the electrode surface forming an inactive film.

From the Fig. 4, i_p / pH findings show that the peak intensity reached the highest value in alkaline solutions. Hence, these media can be employed for analytical use.

In the following part of the work, the applicability of the PG electrode was examined in surfactant-containing solutions. For this purpose, keeping the Vinorelbine concentration at 2.3×10^{-5} M, two kinds of surfactants with opposite charges, namely SDS (anionic) (shown as pink line) and TBAB (cationic) (shown as orange line) were added to BR buffer (pH 2.0-12.0) having a concentration of 5.0×10^{-3} M (Fig 4). When voltammograms in surfactant-free and surfactant-containing solutions were examined, it was observed that the effectiveness of SDS on the peak morphology and intensity of Vinorelbine was very high in acidic solutions (pH 2.0-6.0). On the other hand, the peak potentials shifted to less positive values in this media. Against this, in acidic solutions (pH≤5.0) containing TBAB, the peak intensity was much lower than in solutions without surfactant. In the case of this surfactant, it was observed highest peak intensity at pH 9.0 and 10.0. There was no significant change in peak potentials when cationic surfactant is used.

Since there is one hydroxyl group and four nitrogen-containing regions on the Vinorelbine (Figure 1), this molecule probably should have five pK_a values. However, in aqueous solutions the protonated and nonprotonated structures of the *Vinca* alkaloids (including Vinorelbine) have not been fully elucidated until now. This may be due to the difficulty of determining their pK_a values specific to nitrogen regions. In the Drugbank database **[34]**, the predicted pK_a values of Vinorelbine have been reported as 10.87 (strongest acidic) and 8.66 (strongest basic). Based on the above information, it could be expected that Vinorelbine molecule will exist fully positively charged, mixture of neutral (lipophilic)/positively charged, and neutral species depending on the pH of the environment.

On the other hand, in aqueous solutions containing anionic (in our case SDS) or cationic (in our case TBAB) surfactants below their critical micelle concentrations ($CMC_{SDS} = 8.2\times10^{-3}$ M **[35]**, CMC_{TBAB} = 0.25 M **[36]**), the long hydrophobic tails of their surface micelles can be adsorbed on hydrophobic surface of substrate (in our case PG surface). Thus, they form negatively or positively charged hydrophilic films oriented towards the water bulk phase. Therefore, in strongly acidic solutions containing SDS, the peak intensity was very high due to the electrostatic interaction between the cationic Vinorelbine molecules and the negatively charged head groups of the SDS micelles on the electrode surface. As the acidity of the solutions decreased, the positive charge of the Vinorelbine molecule gradually decreased, thus the peak intensity decreased. If the positively charged surfactant, TBAB, was added to acidic solutions, a decrease in the peak intensity of the Vinorelbine molecule was observed compared to the solutions without surfactant, since the electrostatic forces would work in the opposite direction. The increase in peak intensity of neutral form of Vinorelbine (partially or completely) in TBAB-containing-solutions at pH, 9.0 and 10.0 can be explained by a different mechanism called coadsorption (surface solubilization) **[37-39]**. Reported studies have shown that this mechanism usually takes place in the presence of cationic surfactant with long hydrophobic tail.

When surfactant-free and surfactant-containing solutions were examined, the highest peak currents were reached at pH 2.0 in the presence of SDS, at pH 10.0 in the presence of TBAB, and at pH 12.0 without surfactant. It should be underlined at this point that one of these three media at different pH values could be selected for the determination of Vinorelbine in order to reduce or eliminate the effect of interference from real samples. Considering the effect of the surfactant concentration on the electrode response (will be given later), it was decided to work using BR buffer containing TBAB in the continuation of the work.

3.2.2. Effect of accumulation conditions and other operating parameters

Taking into account the adsorptive properties of Vinorelbine on the PG surface, it was examined the influence of accumulation parameters (accumulation period of time, t_{acc} and accumulation potential, E_{acc}) for 5.5×10^{-6} M Vinorelbine in BR buffer solution containing 5.0×10^{-3} M TBAB (data not shown). The effect of t_{acc} on the analytical signal was investigated in the range of 0-210 s by keeping the E_{acc} value constant at 0.0 V. It was observed a linear increase in the peak current of Vinorelbine in the range of 0-120 s. This value almost remained stable beyond this range. After fixing the t_{acc} at 120 s, the effect of E_{acc} was also investigated over the potential range from 0.0 to +0.6 V. In this case, the oxidation peak current decreased as this parameter increased. After these findings, further experiments were carried out in the continuation of the study by applying E_{acc} of 0.0V for 120 s.

The attention was finally turned to optimize SWV operating parameters such as frequency (f = 5-45 Hz), step potential (ΔE_s = 1-13 mV) and pulse amplitude (ΔE_{sw} = 10-70 mV) under the above conditions (data not presented). To obtain highest sensitivity and better peak shape, the optimized values were: f, 30 Hz; ΔE_s, 11 mV; and ΔE_{sw}, 40 mV.

3.2.3. Effect of surfactant concentration

After optimizing the adsorption and instrumental variables in previous sections, finally the effect of TBAB concentration was investigated in order to provide the best experimental condition for the analytical study of Vinorelbine. By keeping the Vinorelbine concentration constant at 5.5×10^{-6} M, TBAB in the concentration range of 1×10^{-4} M - 1×10^{-2} M was added to the BR buffer (pH 10) solution and the SW-AdSV curves were recorded. As can be seen in Fig. 5, the oxidation peak current of Vinorelbine increased with TBAB concentration up to 3×10^{-3} M. At higher concentration above 3×10^{-3} M, it started to decrease. As a result, the experiments in the analytical examination were carried out by keeping the TBAB concentration constant at 3×10^{-3} M. When this surfactant concentration was present in solution, Vinorelbine signals were increased by approximately 50% compared to solutions without surfactant.

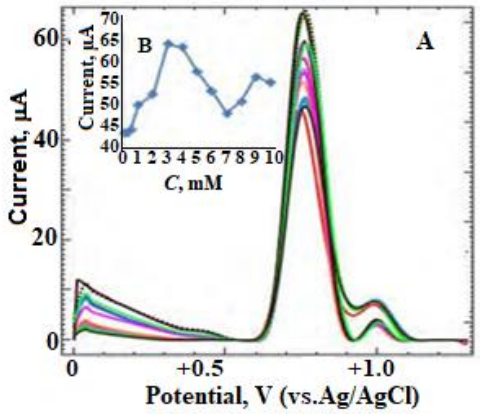

Figure 5. SW-AdSV curves of 5.5×10^{-6} M Vinorelbine recorded in BR buffer (pH 10) containing various concentrations of TBAB (1×10^{-4} M - 1×10^{-2} M). Electrode, PG; accumulation potential 0.0 V; accumulation period of time, 120 s; SW variables: step potential, 11 mV; frequency, 30 Hz; pulse amplitude, 40 mV. Black line, 0 mM TBAB; dashed line, 3 mM TBAB. Inset: plots of i_p vs. the concentration of TBAB.

3.3. Analytical application in aqueous-surfactant solutions

We should first point out that, there is no study on the quantification of Vinorelbine by voltammetric method in the literature so far.

Using the chosen experimental and instrumental conditions presented above, analytical applicability of PG electrode in combination with SW-AdSV was tested in 3×10^{-3} M TBAB-containing BR buffer at pH 10 by plotting the peak currents as a function of Vinorelbine concentration. The relevant stripping voltammograms and corresponding calibration graph are given in Figure 6.

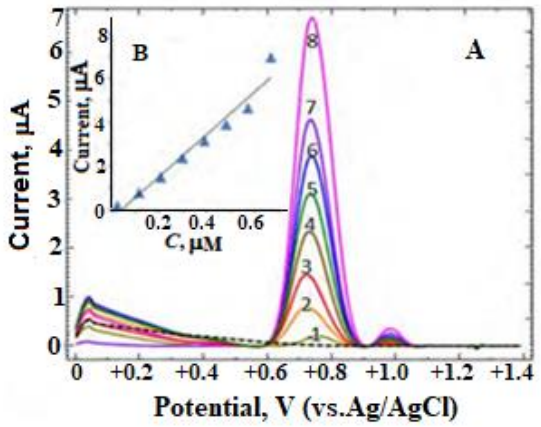

Figure 6. SW-AdSV curves of for Vinorelbine concentration levels of (1) 0.023, (2) 1.15, (3) 2.07, (4) 2.99, (5) 3.91, (6) 4.83, (7) 5.75, (8) 6.67 µM recorded in BR buffer (pH 10) containing 3×10^{-3} M TBAB. Dashed line, supporting electrolyte. Inset: plots of i_p vs. the concentration of Vinorelbine. The other operating conditions are as shown in Fig. 5.

It was obtained a linearity between the oxidation peak currents on the Vinorelbine concentration from 2.3×10^{-8} to 5.8×10^{-6} M (as base form, 17.9 – 4518 ng mL^{-1}). The SWV response at a potential of +0.75 V increased proportionally with the concentration of Vinorelbine (Fig. 6, inset) to present a highly linear calibration plot according to the following equation:

i_p (µA) = 0.0097 C (nM) – 0.4013 (r = 0.997, n = 7)

In the equation, i_p represents the oxidation peak current, C the Vinorelbine concentration, r the correlation coefficient and n the number of experiments, respectively.

Limit of detection (LOD) and quantification (LOQ) values obtained with the aid of analytical curves were calculated according to the 3 s/m and 10 s/m, respectively. In this equation, s is the standard deviation of the peak currents (three runs) of the lowest concentration of the relevant linearity range (2.3×10^{-8} M) and m *is* the slope of the used calibration curves. The values were calculated as 7.5×10^{-9} M (as base form, 5.8 ng mL^{-1}) and 2.5×10^{-8} M (as base form, 9.47 ng mL^{-1}) for LOD and LOQ, respectively.

As can be seen from the values found, PG allowed the detection of very low concentrations of Vinorelbine without applying any chemical modification to the electrode (except for a simple electrochemical pretreatment). Electrochemical pretreatment used in this work is very fast, simple and cost-effective compared to other surface modification strategies and is environmentally friendly as it does not require any toxic chemicals. According to the opinion of some researchers, the increased electrochemical response obtained on the PG electrode compared to other electrode materials is due to the clay material in its structure **[23]**. Since this material provides a porous structure and a special surface area for the PG electrode, it increases the electrocatalytic efficiency of the electrode in the redox reactions of many analytes.

The analytical performance of the voltammetric method (in terms of sensitivity) developed by our working group for the first time is comparable to some of the separation methods using MS or MS/MS detection system **[6,7]**.

In order to evaluate the precision of the here-reported voltammetric method developed for Vinorelbine, solutions at a concentration of 3.5×10^{-7} M were prepared under the selected experimental conditions and stripping voltammograms of these solutions were recorded nine times in the same day. Oxidation peak current and potential values were measured from these voltammograms and the values found were evaluated as intra-day repeatability. According to the results, the RSD values were calculated as 1.23% (for peak current) and 0.32% (for peak potential).

3.3.1. Analysis of real samples

To verify the performance characteristics of the developed method in real samples, its applicability was first tested in commercially pharmaceutical formulation (injectable solution). The vial solutions were easily prepared after a simple dilution described in Section 2.4. By comparing the voltammograms of the vial content with the voltammograms of the standard Vinorelbine, it was observed that the both curves were compatible. Considering the required dilutions of the vial sample, Vinorelbine content was calculated as 51.50 mg (71.33 mg as ditartrate salt) per vial (RSD of 2.97%). This value is in good agreement with the label value of 50 mg per vial declared by the producer (recovery, 103.0%). In order to evaluate the validity of the proposed method, recovery experiments were applied by adding known volumes of standard Vinorelbine solutions to the previously analyzed sample solution between the linearity range. It was obtained the satisfactory recovery from 93.0 to 107.0%. It presents that there is no remarkable interference in the commercial pharmaceutical injectable forms.

Due to the high sensitivity and reproducibility of the voltammetric method developed on a disposable PG electrode were also investigated for the determination of Vinorelbine in spiked human urine sample without any complex separation. The only treatment was a simple dilution (1:200 v/v) with the selected supporting electrolyte. At the beginning of the experiments,

voltammograms of blank urine samples were prepared in supporting electrolyte (BR buffer, pH 10.0) in the absence and presence of TBAB. No oxidation steps were observed for biomolecules (uric acid, ascorbic acid, dopamine) in the potential range where the Vinorelbine signal is observed in surfactant-free solutions. In a previous study reported by our working group using SW-AdSV technique on PG electrode [40], the uric acid peak in the urine sample diluted with BR buffer (pH 10.0) appeared at approximately +0.30 V. The oxidation steps of ascorbic acid and dopamine on the PG electrode generally occur at lower positive potential values compared to uric acid [41,42]. On the other hand, when the same experiments were repeated in TBAB-containing solutions, a peak with a low intensity was observed +0.6 and +0.8 V. It can be assumed that this peak may be related to a different urine component, which is in anionic form at the pH studied and thus interacts with the cationic surfactant. Since standard addition method will be used for this purpose, it is clear that this peak will not affect the results. Considering the high peak intensity and smooth peak morphology of Vinorelbine in surfactant-free solutions at this pH, in order to avoid any risk, the studies in urine samples were performed in BR buffer pH 10.0 without surfactant. Analysis of model human urine sample (containing spiked concentration of 2.0×10^{-7} M for Vinorelbine in the electrochemical cell) is illustrated in Fig. 7A with the graphical evaluation of multiple standard addition method. The oxidation signal at about +0.70 V increased proportionally after five consecutive additions of the standard Vinorelbine solutions (Fig. 7B), yielding a linear calibration plot; i_p (μA) = 8.8263 C (μM) + 1.9281 (r = 0.987).

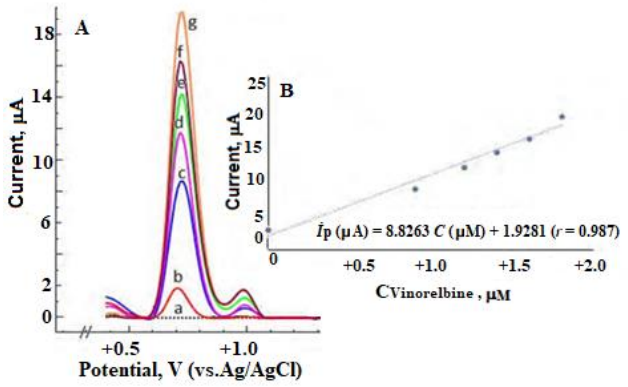

Figure 7. **(A)** SW stripping voltammograms recorded in the model urine sample from volunteer (diluted with BR buffer, pH 10 in the ratio of 1:200, v/v). Dashed lines (a) present the voltammogram of unspiked sample of urine; (b) in the presence of 2×10^{-7} M Vinorelbine; (c–g) after standard additions of 45, 60, 70, 80 and 90 μL from 2×10^{-4} M Vinorelbine). **(B)** The result of analysis by the standard addition method. The other operating conditions are as shown in Fig. 5.

As shown in Table 1, the quantification of Vinorelbine presented satisfactory RSD value and recovery indicating that the proposed method could also accurately determine Vinorelbine from urine samples.

Table 1. Analysis of model urine sample for Vinorelbine by SW-AdSV developed in this study using PG electrode.

Added (μM)	Found[a] (μM)	Recovery[b](%)± % RSD (%)
0	Not detected	
0.2	0.218	109.0± 4.58

[a]Calculated by using standard addition method. Values reported are mean of three different independent analyzes of the same sample. [b]Calculated as: (found concentration/added concentration) × 100.

4. CONCLUSION

As stated in the Introduction Section, there is very limited number of studies for the voltammetric determination of *Vinca* alkaloids. To our knowledge, only one electrochemically based research, which is quite old, has been reported for Vinorelbine. In the related study, no study was conducted for the determination of this compound, only the electrode mechanism was tried to be clarified on GC surface. In our present work, it was first shown how the Vinorelbine molecule is oxidized on PG electrode in aqueous or aqueous-surfactant (anionic/cationic) solutions. Then, voltammetric method was developed for the determination of real samples.

The observation obtained from the findings can be important in two respects: (*i*) Considering that the redox mechanism of Vinorelbine and similar compounds is very complex (especially in terms of forming free radicals and intermediate), the information obtained from this work may shed a great deal of light on the oxidation studies of *Vinca* alkaloids in the surfactant environment. (*ii*) The voltammetric method developed in this study will provide an alternative to chromatographic methods, which are often used for their determination.

It can be assumed that the proposed voltammetric method using a disposable, economical and environment-friendly PG electrode may also constitute the first step for on-site measurements of not only Vinorelbine but also other *Vinca* alkaloids, especially in wastewater around the hospital. The LOD value reached in the presence of surfactant looks very promising for future research.

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