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Osteryoung square wave stripping voltammetry at mercury film electrode for monitoring ultra trace levels of Tarabine PFS and its interaction with ssDNA

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Abstract

The electrochemical oxidation and reduction behaviour of adsorbed species of antimetabolic antineoplastic agent Tarabine PFS (Cytosar-U) in Sorensen buffer solution of different pH values at an in situ-mercury film electrode (MFE) is studied using cyclic voltammetry (CV) and Osteryoung square-wave stripping voltammetry (OSWSV). Optimal experimental and operational parameters have been selected for the drug preconcentration and determination in aqueous medium. Based on the adsorption and accumulation of Tarabine PFS using Osteryoung square-wave anodic stripping voltammetry (OSWASV) at MFE, the drug is easily detected as 0.134 ng/ml (5.51×10^{-10} M). Calibration plots have been constructed at different accumulation times. The standard deviation ($n = 10$) at a concentration level of 6×10^{-8} M Tarabine PFS is 0.062. The interaction of ssDNA with the drug under the optimal conditions at pH 7.7 has been studied. The formal potentials E° and $E^{\circ\prime}$ and the equilibrium constants K_1 and K_2 have been calculated for the free form of Tarabine PFS and the bonded form with ssDNA, respectively. It was found that K_2 value for the bonded oxidized form is 298 times than that of K_1 for the bonded reduced form. Therefore, ssDNA has been found to interact strongly with the oxidized form of the drug. The method has been used for the nanogram determination of ssDNA with 1.9% variation coefficient. Detection limit of 3 ng/ml ssDNA has been achieved. Possible interfering organic compounds, cations and anions have been tested. The method has been applied for the drug determination in urine samples, down to 0.23 ng/ml could be easily achieved in such samples.

Keywords: Tarabine PFS; DNA; In situ MFE; Determination; Urine analysis

1. Introduction

Tarabine PFS (Cytosar-U) is the most important antimetabolic antineoplastic agent used in the therapy of acute myelocytic leukemia [1]. It is the single most effective agent for induction of remission in this disease. The drug is particularly useful in acute

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non-lymphocytic leukemia in adults and is more effective when used with other agents, particularly anthracyclines or mitoxantrone. The drug also is used in combination with other chemotherapies to treat non-Hodgkin's lymphomas in adults and in children [2].

This compound is noteworthy structurally in that its arabinose moiety is epimeric with ribose at the 2′-position. After anabolism to the triphosphate, this modification inhibits the conversion of the cytidylic acid to 2′-deoxycytidylic acid. Furthermore several studies have reported that persons working with cytostatic drugs (Tarabine PFS), e.g. clinical and pharmaceutical staff, show a marginally higher frequency of DNA damage [3–6]. These chromosomal aberrations were classified as possibly precancerous cell degeneration. Studies now indicate that there is a relationship between inhibition of DNA and the total amount of Tarabine PFS incorporated into DNA. Thus, the incorporation of about five molecules of this compound per 10^4 bases in DNA decreases cellular clonogenicity by about 50% [7]. There is also evidence that the drug incorporated into DNA slows DNA template function [7]. Tarabine PFS is degraded in the gastrointestinal tract, after oral administration, only about 20% of the drug reaches the circulation and less than 10% of the injected dose is excreted unchanged in the urine within 12–24 h.

Several analytical methods for the determination of Tarabine PFS have been reported including UV-spectrophotometry and polarography [8–12], derivative spectroscopy [13,14], gas chromatography [15], high performance liquid chromatography (HPLC) [16–22] and capillary electrophoresis techniques [23]. Also, Tarabine PFS and other pyrimidine derivatives have been detected by radio-enzymes [24], radio-immunological [25] and microbiological methods of analysis [26,27].

Polarography and voltammetry can be considered as a convenient alternative to these routinely employed analytical methods. They present the great advantage of permitting a direct, simple and rapid determination that requires a minimum volume of sample. The ideal working electrode should have a favorable electrochemical behavior of the analyte of interest, a reproducible surface area and low background current [28]. Mercury film electrode (MFE) is the most important electrode to achieve these requirements. MFE offers a large surface area to volume ratio that provides high amalgam concentration during the deposition step, also extension for the potential range limit is achieved. As a result, high plating efficiency and high sensitivity were achieved. In addition, mercury films give superior selectivity since the diffusion of analyte from the bulk of the film to the surface is very fast. Although preplated mercury films were used initially, best results were achieved with the in situ plated mercury film described by Florence [29].

Tarabine PFS was determined by DC and differential pulse polarography techniques in 0.2 mol/l KCl and phosphate buffer as supporting electrolyte at pH value 6–7 [30,31]. These studies were limited to a concentration level between 10^{-7} and 10^{-5} mol/l and the reduction peak potential of the compound was very closely to the hydrogen evolution potential. The electrochemical behaviour of the Tarabine PFS has been studied using polarography in phosphate buffer aqueous media [32,33]. These papers were concerned with association properties of the compound and its electrochemical behaviour in the cathodic direction at dropping mercury electrode.

There is a growing demand for the production of a deoxyribonucleic acid (DNA) biosensors with application in medicine, the food industry, agriculture, veterinary science and environmental science. Also, the direct and reliable electrochemical detection of DNA is of paramount importance to the development of modern DNA hybridization chips, for the detection of nucleic acids following their electrophoretic separation, or for the sensing of DNA damage and interactions [34]. This paper reports the use of Osteryoung square-wave stripping voltammetry for monitoring ultra trace levels of ssDNA and its interaction with the anticancer drug Tarabine PFS.

2. Experimental

2.1. Reagents and solutions

A fresh solution of Tarabine PFS was prepared daily in doubly distilled water. The solutions are diluted as required for standard additions for quantitative analysis. Tarabine PFS was provided by Upjohn s.a. company, Puurs-Belgium. It was used without any further purification. Different types of supporting

electrolytes were used, e.g. acetate, borate, phosphate, Britton-Robinson and isotonic Sorensen buffers. The latter was chosen as it gives the best signal, it is prepared by mixing definite weights of Na_2HPO_4 and NaH_2PO_4 at each desired pH value in presence of NaCl to adjust the tonicity to be 0.92 at $25 \pm 1\,^{\circ}C$ [35]. Stock solution of mercuric ion (10^{-2} mol/l) is prepared by dissolving the required weight of basic mercuric nitrate (May & Baker LTD., Dagenham, UK) in doubly distilled water. Single Stranded Calf thymus DNA for Molecular Biology was of Sigma quality (Lot 43H67951). It was prepared (5 mg in 5 ml injection water) by a modification of the method of Alberts and Herrick using calf thymus DNA, D 1501. The resulting solution was divided into ten aliquots (for daily use) and was kept in polyethylene vials frozen. One mg of ssDNA is equivalent to approximately 25 A_{260} units. Urine samples were taken from the hospital after metabolism. All other reagents were of grade quality.

Solutions of diverse ions used for interference studies are prepared using the nitrate, sulphate or perchlorate salts of the metal ions. Dilute acids are added to prevent hydrolysis whenever needed.

2.2. Instrumentation

Voltammetric measurements are recorded using the CV-50 W Voltammetric Analyzer (USA) electrochemical running under windows^{TM}software. All controlled parameters are entered through a BAS/windows interface. This information is transferred to the CV-50W microprocessor where optimum hardware settings are calculated for the specified technique. These values are loaded automatically and upon applying the command run; data is collected and transmitted to the PC where it is displayed in virtual real time. Standard C-2 cell stand is fully shielded in a Faraday cage (EF-1080) with three electrodes: a glassy carbon working electrode (MF- 2012, diameter 3 mm), a silver/silver chloride reference electrode (MF- 2063) and a platinum wire auxiliary electrode (MW-1032). Voltammograms are collected using an hp HEWLETT PACKARD laser jet 4L printer. These data were sometimes plotted using Microsoft Excel program utilizing either the line or scatter presentation.

The pH's were measured using the Fisher Scientific Accument pH Meter Model 810 equipped with a combined glass electrode, which is calibrated regularly with buffer solutions (pH 4.00 and 7.00) at $25 \pm 1\,^{\circ}C$. V3 series HTL micropipettes (Germany) were used to pipette μl volumes of solutions.

2.3. Recommended procedures

A 10 ml volume containing 0.1 M of isotonic Sorensen buffer as supporting electrolyte is added to the cell and degassed with highly purified nitrogen for 8 min. The in situ MFE is prepared by adding a definite volume of basic mercuric nitrate solution directly to the sample solution and simultaneously depositing the mercury and the measured compound [29,36]. Drug molecules are then anodically stripped from the mercury thin film by scanning Osteryoung square-wave positive potential from −1.8–0.0 V at pH 7.7. This electrode combines the sensitivity of thin films with high selectivity and reproducibility. After the analysis the mercury film can be completely removed from the electrode by simply wiping with BAS cloth disk coated with two drops of polishing alumina (CF-1050) and doubly distilled water. The solution is stirred by using stirring magnet (ER-9132) at constant stirring rate (300 rpm). The interaction of DNA with Tarabine PFS is studied by titration of different ssDNA concentrations with fixed concentration of the drug in 0.1 M Sorensen buffer at pH 7.7. All the results are obtained at room temperature.

3. Results and discussion

Preliminary investigations of the electrochemical behaviour of Tarabine PFS at various types of working electrodes viz. Static mercury drop electrode (SMDE), carbon paste electrode (CPE), glassy carbon electrode (GCE) and MFE, indicate that well- defined and well-shaped peaks have appeared onto in situ mercury film electrode (Fig. 1). The peak height at about −1.09 V using the MFE is four and twenty five times greater than the GCE and the SMDE, respectively. This is due to the large surface area to volume ratio (about three times greater than SMDE) and the strong adsorption character of compound at the MFE surface. So, the latter was the preferable electrode to all further experiments.

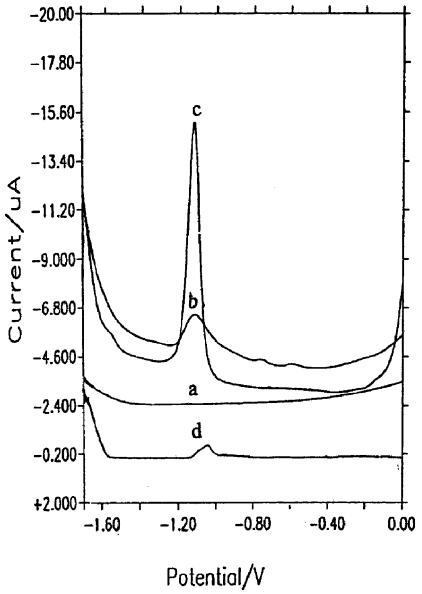


Fig. 1. Osteryoung square-wave adsorptive stripping voltammograms (OSWASV) of: (a) 0.1 M Sorensen buffer and 0.073 NaCl at MFE; (b) $a+2\times10^{-6}$ M Tarabine PFS at GCE; (c) $a+2\times10^{-6}$ M Tarabine PFS at MFE and (d) $a+2\times10^{-6}$ M Tarabine PFS at SMDE after 60 s accumulation time at pH 7.7.

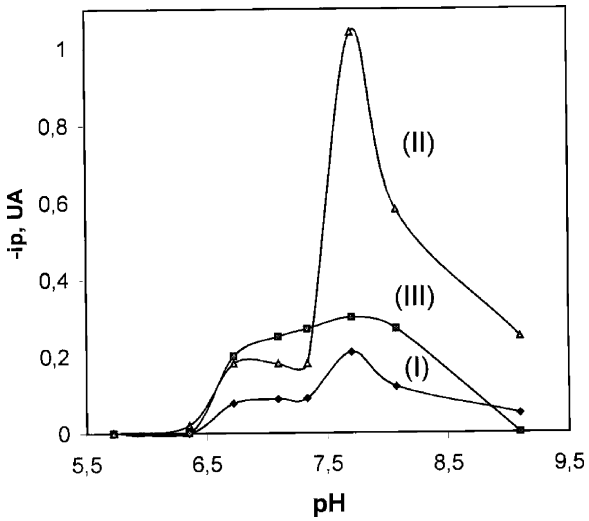
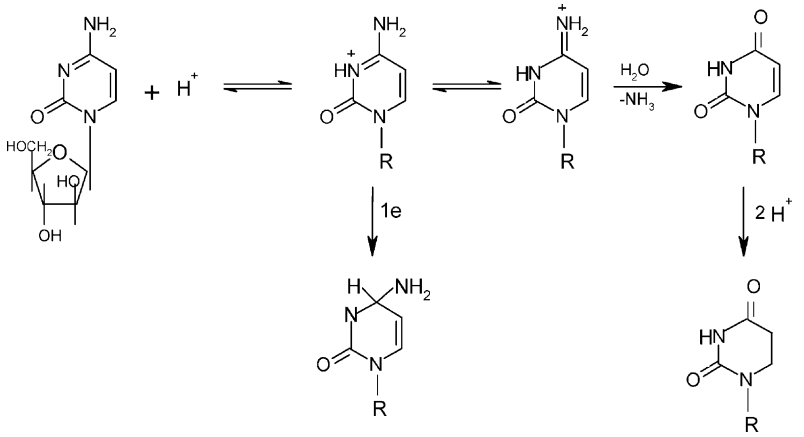


Fig. 2. Effect of the pH on i_p of 1×10^{-6} M Tarabine PFS at MFE in 0.1 M isotonic Sorensen buffer. Peaks (I, II) are the oxidation peaks of the drug and peak (III) is the reduction one.

3.1. Electrochemical behavior of Tarabine PFS

Voltammetric behavior of Tarabine PFS in solutions with different pH values was carried out in 0.1 M isotonic Sorensen buffer at MFE. OSWSV mode with high scan rate was used to enhance the Tarabine PFS adsorption current relative to the diffusion controlled one. The square-wave voltammograms of 1×10^{-6} M Tarabine PFS in the pH range 2.6–9.1 show two oxidation peaks (peaks I and II) and one reduction peak (peak III). Fig. 2 indicates the effect of the pH on i_p values of peaks (I, II and III). Below pH 5.72 the peaks are ill defined and two well separated oxidation peaks are appeared at pH values higher than 5.72. The first oxidation Peak (I) is located at about −1.44 V, its shape and height is largely dependent on the concentration of Tarabine PFS and the accumulation time. As it is cited in the literature, in aqueous solutions Tarabine PFS takes an amino form that is in equilibrium with the protonated form [33]. So, the dependence of the peak potential on the pH values supports that there is no proton consumption and therefore this peak may be attributed to strong adsorption character of the protonated form of Tarabine PFS at MFE surface. The second oxidation peak (II) is more pronounced than the first one, it is appeared at about −1.01 V at pH 6.35 and shifted to more electronegative potentials with increasing pH values indicating the involvement of proton consumption during the electrochemical process. The best morphology is achieved at pH 7.7, the height of the peak is then decreased with increasing the pH values until it is completely disappeared after pH 9.02, this is probably due to the complete hydrolysis of the drug in alkaline media. Therefore, we postulate that appearance of the second oxidation peak in weakly acidic and neutral media is due to the oxidation of $-NH_2$ group in pyrimidine ring of Tarabine PFS [37]. The reduction peak (III) is located at about −1.05 V, pH 6.72 and then disappeared completely in alkaline media (above pH 9.0, Fig. 2). This peak may be attributed to the reduction of the adsorbed oxidized form at C(5)=(6) double bond forming dihydro derivative [37] or the reduction of N(3)=C(4) double bond as stated by Teijeiro and Marin [33]. Therefore, it is concluded that the electrode mechanism is identical to that of the catabolism reaction of pyrimidines [37] illustrated by the following scheme:



3.2. Cyclic voltammetric measurements

The nature of the electrochemical process has been carried out applying cyclic voltammetry technique at different pH values. Below pH 5.5, the peaks of Tarabine PFS are ill defined. One oxidation peak at about −1.09 V and one reduction peak at about −1.15 V are obtained at pH 7.7 with scan rate of 100 mV/s and then these peaks completely disappeared above pH 9.0. The effect of scan rate has been tested on 8×10^{-5} M Tarabine PFS, pH 7.7 at the MFE (Fig. 3). The irreversibility of the electrochemical behaviour for the drug increases with increasing scan rate values. The relationship between scan rate and the peak height gave a linear relationship with slope value tends to unity ($0.93\,\mu A\,s\,V^{-1}$) and correlation coefficient value (0.9986) indicating an adsorption behavior for the drug. The peaks also decreased rapidly upon repetitive scans with a little shift in potentials, indicating fast desorption from the electrode surface. Therefore, rapid scan rates are required for such measurements.

3.3. Analytical aspects at the MFE

At pH 7.7, the effect of different experimental parameters viz. wave form, amplitude, frequency, stirring rate ... has been tested. Fig. 4 shows a comparison between linear sweep stripping voltammetry (LSSV) curve a, differential pulse stripping voltammetry (DPSV) curve b and Osteryoung square-wave stripping voltammetry (OSWSV) curve c. Its obvious that OSWSV and DPSV are much sensitive than LSSV with about six-fold increment for the former one. Therefore, further investigations have been made using OSWSV technique. The effect of the in situ mercury film (mercuric ion concentration) on the determination of Tarabine PFS has been tested. It was found that the best sensitivity and reproducibility of the drug oxidation peak is achieved in the presence of 11 times of mercuric ion concentration greater than drug concentration. So, this ratio was chosen for complete adsorption of the drug species. The thickness of the mercury-film disk electrode (ℓ) was then

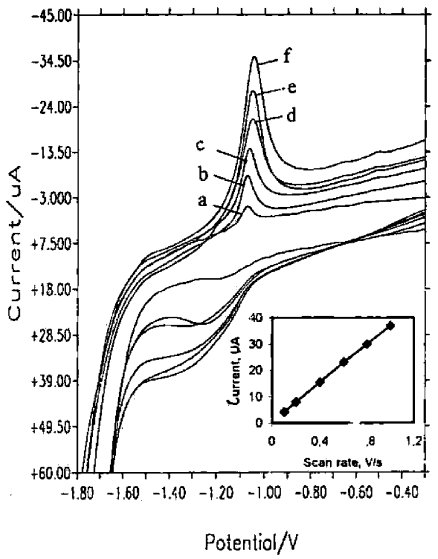


Fig. 3. Effect of scan rate on the cyclic voltammograms of 8×10^{-5} M Tarabine PFS at MFE surface in presence of Sorensen buffer, pH 7.7. Scan rate: (a) 0.1; (b) 0.2; (c) 0.4; (d) 0.6; (e) 0.8 and (f) 1.0 V/s.

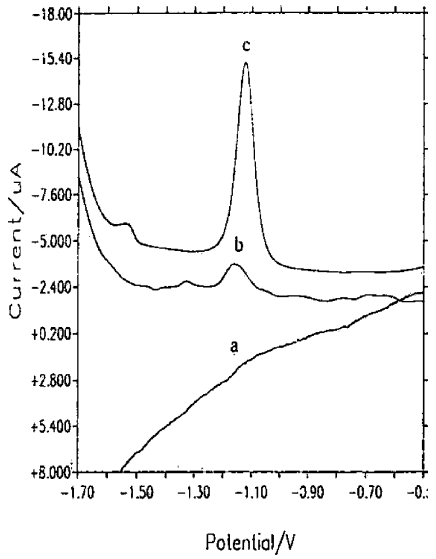


Fig. 4. Comparison between LSSV (a), DPSV (b) and OSWSV (c) of 8×10^{-7} M Tarabine PFS in 0.1 M Sorensen buffer + 0.073 M NaCl + 8.8×10^{-6} M Hg^{2+}, pH 7.7. All parameters of each mode are cited in text.

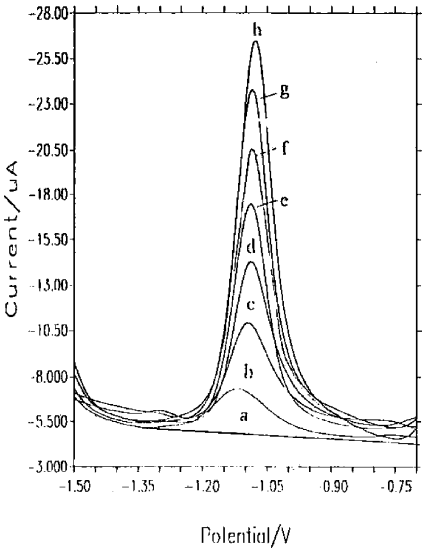


Fig. 5. Effect of the accumulation time on the OSWAS voltammograms of 4×10^{-8} M Tarabine PFS at pH 7.7: (a) blank; (b) 0; (c) 30; (d) 60; (e) 120; (f) 180; (g) 240 and (h) 300 s.

calculated from the following equation [28]:

$$\ell = \frac{2.43it}{r^2}$$

to be 35 nm, where i the mercury ion deposition current, t the deposition time and r the disk radius. Other experimental parameters were optimized by varying each one.

Under the foregoing optimal parameters, the adsorptive preconcentration of Tarabine PFS at MFE surface and the application of a subsequent square-wave scan in the positive directions gave rise to two oxidation peaks in Sorensen buffer, pH 7.7. A large response after 60 s of accumulation time for an assay concentration of 4×10^{-7} mol/l Tarabine PFS is obtained greater than the direct response ($t = 0$). This indicated that Tarabine PFS could be determined strongly using adsorptive square-wave stripping voltammetry (AdSWSV) compared to direct square-wave voltammetry (SWV). The effect of accumulation time (t_{acc}) on the stripping peak current of 4×10^{-8} mol/l (9.73 ng/ml) Tarabine PFS has been investigated (Fig. 5). The linearity limits of time at different concentrations of Tarabine PFS are studied. Linear relationships are obtained up to 300 s in presence of 1.46 ng/ml Tarabine PFS; it decreased to be 120 s in presence of 9.72 μg/ml (4×10^{-5} M) Tarabine PFS.

The charge of the double layer (Q_{dl}) is calculated by applying a double potential-step chronocoulometric technique for the supporting electrolyte at pH 7.7. In the presence of 6×10^{-6} M Tarabine PFS, the adsorbed charge values (Q_{ads}) has been calculated and found to be 9.8 μC. The surface coverage can be measured from the division of the number of coulombs transferred by the conversion quantity (nFA) yielding coverage of 8.98×10^{-11} mol cm^{-2}. Thus, every mole Tarabine PFS occupies an area of 4.764 nm^2 at the electrode surface.

3.4. Quantitative Utility

Fig. 6A shows the voltammograms for solutions of increasing Tarabine PFS concentrations in presence of 0.1 M isotonic Sorensen buffer applying 120 s accumulation time at pH 7.7. Well-defined stripping peaks were observed. The resulting calibration plots are also shown in Fig. 5B following 0 (a), 30 (b), 60 (c), 120 (d) and 240 (e) seconds of preconcentration. The stripping peak currents for the drug increase linearly with increasing their concentrations up to 10 μM at lower accumulation times (Fig. 6B, curve b, c) and up to

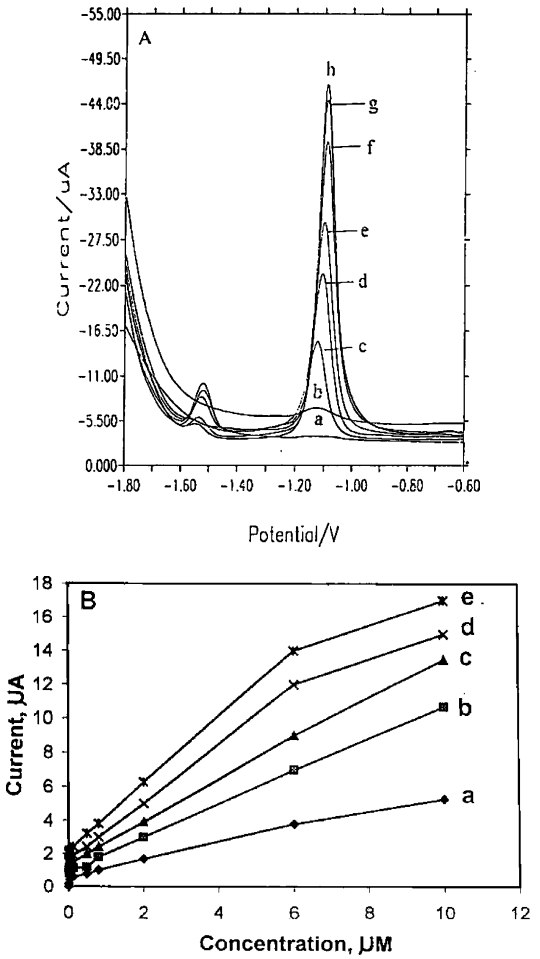


Fig. 6. (A) Standard addition of Tarabine PFS at MFE after 120 s accumulation time at pH 7.7 in presence of 0.1 M Sorensen buffer and 0.073 NaCl (a), accumulation potential −1.8 V: (b) 5×10^{-9} M, (c) 1×10^{-8} M, (d) 4×10^{-8} M, (e) 1.2×10^{-7} M, (f) 4.8×10^{-7} M, (g) 8×10^{-7} M and (h) 2×10^{-6} M. (B) Calibration plots of different Tarabine PFS spikes from 5 nM to 10 μM at the MFE applying different accumulation times: (a) 0 s; (b) 30 s; (c) 60 s; (d) 120 and (e) 240 s, other parameters as cited in the text.

6 μM at higher ones (curve d, e). Other slopes are obtained in higher concentration ranges at 120 (curve d) and 240 (curve e) accumulation times probably due to reorientation of the drug molecules adsorbed on the surface of MFE. The calculations of these calibration plots are summarized in Table 1.

The adsorptive accumulations of Tarabine PFS result in highly reproducible stripping peak currents. For ten successive measurements of 0.015 μg/ml Tarabine PFS (6×10^{-8} M) applying 120 s accumulation, the mean peak current was 0.67 μA with standard deviation 0.062. A detection limit of 0.24 ng/ml Tarabine PFS (1×10^{-9} M) is easily achieved using the foregoing optimal conditions applying 240 s accumulation. Also, the detection limit was theoretically calculated using the equation $dL = 3S.D./a$ [38] where S.D. is the standard deviation and a the slope of the calibration curve. It was found to be 0.134 ng/ml Tarabine PFS (5.51×10^{-10} M). This dL value is more sensitive than that cited in other publications [30,31] indicating the high sensitivity of our procedure.

3.5. Determination of ssDNA and its reaction with Tarabine PFS

The interaction of the anticancer drug Tarabine PFS with ssDNA has been studied under the foregoing experimental conditions. The acidic and alkaline media have been avoided to prevent any denaturing of DNA molecule. Fig. 7 shows the OSWS voltammograms of 0.1 M isotonic Sorensen buffer as supporting electrolyte (peak a) in presence of 0.243 ng/ml Tarabine PFS (peak b). Peak c is obtained by adding 1 μg/ml of ssDNA in absence of Tarabine PFS. The effect of the spiked ssDNA on the adsorptive square-wave stripping voltammogram of Tarabine PFS is indicated by peak d after 120 s accumulation time. It is observed that one-half reduction in the Tarabine PFS peak height is obtained with a little shift in potential.

The electrochemical behaviour of ssDNA molecules was studied by cyclic voltammetry at pH 7.7 at MFE and indicated that there is one oxidation peak at about −1.1 V and no cathodic peak under the applied potential range. The electrochemical inactivity of DNA in the cathodic potential range may be due to the merging of cathodic peaks at low ssDNA concentrations with the background discharge [39]. So, the electrochemical reaction of ssDNA at the surface of MFE is considered to be irreversible reaction. The oxidation mechanism of ssDNA molecule is probably due to the oxidation of $-NH_2$ group in the guanine molecule [40], it cannot be due to the oxidation of adenine molecule because the guanine groups are initially oxidized and this is proved by several methods [41]. Also, the cyclic voltammograms of guanosine 5′-monophosphate solutions give the same behaviour at the same potential, which proved the suggested mechanism for the

Table 1
Characteristic features of the calibration graphs for Tarabine PFS at the MFE, pH 7.7 in 0.1 M isotonic Sorensen buffer

Concentration range (M)	t_{acc} (s)	Equation ($\mu A\,\mu M^{-1}$)	Limit of linearity (M)	Standard deviation	Correlation coefficient
5×10^{-9}–1×10^{-5}	0	$Y = 0.39X + 0.2$	1×10^{-5}	1.7167	0.9996
5×10^{-9}–1×10^{-5}	30	$Y = 0.55X + 0.4$	1×10^{-5}	1.8278	0.9989
5×10^{-9}–1×10^{-5}	60	$Y = 0.62X + 0.6$	1×10^{-5}	1.8823	0.9981
5×10^{-9}–1×10^{-5}	120	$Y = 0.69X + 0.7$	6×0^{-6}	1.9131	0.9978
5×10^{-9}–1×10^{-5}	180	$Y = 0.71X + 0.8$	6×0^{-6}	1.9291	0.9974

oxidation of ssDNA molecules. The experimental results of the relationship between the square root of scan rate and the peak height gave a linear relationship with slope value tends to unity (0.97 $\mu A\,mV^{-1/2}\,s^{1/2}$) and correlation coefficient value (0.9986). Therefore, it is assumed that the appearance of the oxidation peak is due to the adsorption of the oxidized guanine group.

The interaction of ssDNA macromolecule with the anticancer drug-under investigation has been studied by adding ssDNA concentrations to definite concentration of the drug and running voltammograms in the anodic direction of potentials without preconcentration (i.e. at 0 s) and with several preconcentration times (i.e. at 30, 60 and 120 s). Fig. 8A shows the standard additions of ssDNA on 1×10^{-6} M Tarabine PFS at 120 s preconcentration time. The oxidation peak (I) height is decreased by adding ssDNA concentrations without shifting in potential. This depression in peak (I) currents may be due to only the adsorptive competition between ssDNA molecules and the protonated form of the drug at the electrode surface. There is also a depression in the height of peak (II) by adding different concentrations of ssDNA. This depression

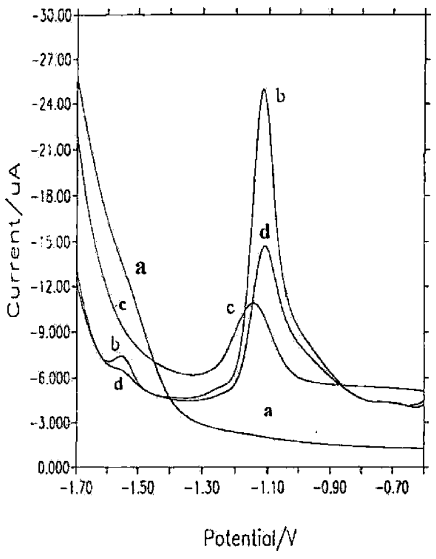


Fig. 7. Osteryoung square-wave anodic adsorptive stripping voltammograms of: (a) supporting electrolyte; (b) $a + 1$ nm/ml Tarabine PFS; (c) $a + 1\,\mu g$/ml DNA and (d) $a + b + c$ at pH 7.7 using the experimental parameters.

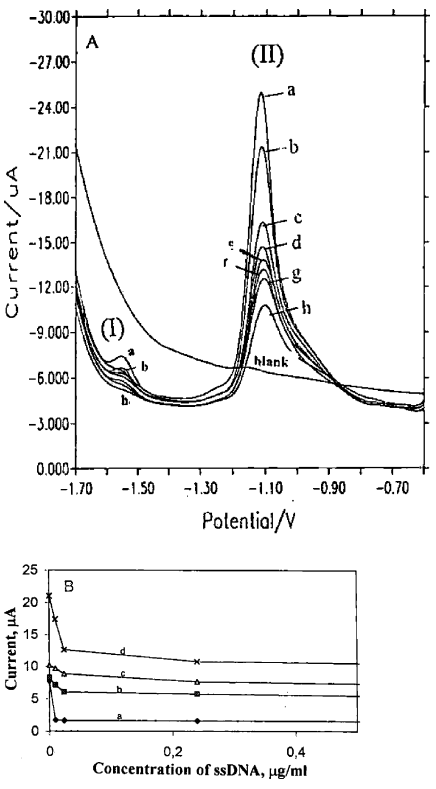


Fig. 8. Standard addition of different concentrations of ssDNA ranged from 9.7 ng/ml to 4.0 μg/ml on 1×10^{-6} M Tarabine PFS at 120 s (A) and at different preconcentration times (B): (a) 0; (b) 30; (c) 60 and (d) 120 s.

is highly observed without preconcentration (Fig. 8B, curve a) with adding lower concentrations of ssDNA molecules and it is observed to be decreased by increasing the accumulation times (curve b–d). As it is well-known that the interactions between the purine or pyrimidine micro molecules and the DNA bases may be due to Van der Waals or hydrogen bonding between certain groups on the drug molecules and on the DNA bases or ionic interactions [1]. So, we postulated that the mechanism of the interaction of ssDNA with Tarabine PFS is that the formation of hydrogen bond between the –C=O group in the oxidized form of the drug and $–NH_2$ group in guanine moiety. This postulate is probably predominant especially when we use ssDNA molecules. The existence of weak shift in the potential values may be due to the formation of hydrogen bonding between ssDNA and the drug. This is also proved by calculating the formal potential [42] for Tarabine PFS in absence of DNA (E°) and in presence of DNA ($E^{\circ\prime}$) using cyclic voltammograms of both at 400 mV/s. It was found that E° and $E^{\circ\prime}$ values are −1.173 and −1.1 V. This positive shift in formal potential (about 73 mV) proves that the interaction of DNA with the oxidized form of Tarabine PFS is stronger than that with the reduced form. We could then calculate the ratio of the equilibrium constant (K_1) between the reduced free form and the reduced bonded form and the equilibrium constant (K_2) between the oxidized free form and the oxidized bonded form by applying the following equation [42]:

$$2(E^{\circ\prime} - E^\circ) = 0.059 \log\left(\frac{K_2}{K_1}\right)$$

It was found that K_2 value is 298 times than that of K_1. This means that ssDNA is completely interacted with the oxidized form that contains carbonyl group and approximately negligible interaction with the reduced form that contains amino group. Therefore, it is concluded that the carbonyl group—in the oxidized form—is the predominant function group for the interaction between ssDNA and Tarabine PFS. This kind of interaction has been proposed previously using other methods, e.g. UV hyperchromicity and 1HNMR spectroscopy or single cell gell electrophoresis [43,44].

The use of adsorptive stripping Osteryoung square-wave voltammetry for monitoring ultra trace levels of ssDNA under the foregoing experimental conditions has been also utilized. Fig. 9 shows the effect of accumulation time on the oxidation peak of 25 ng/ml ssDNA at pH 7.7. The peak height increases linearly with increasing the preconcentration time up to 300 s. Because of the oxidation peak potential of ssDNA is very close to the oxidation peak potential of Tarabine PFS, the calibration plots of ssDNA (Fig. 10)

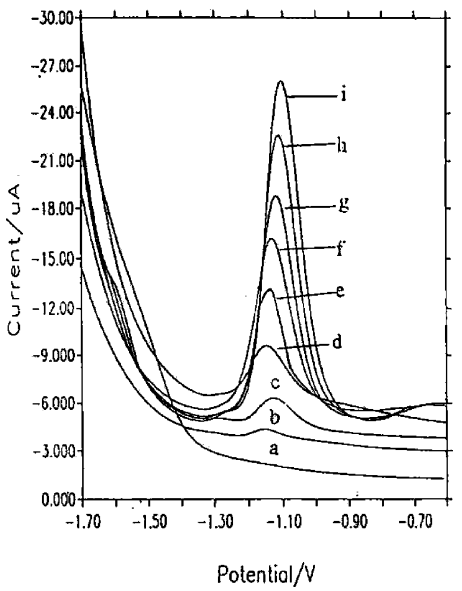


Fig. 9. Effect of the accumulation time on the oxidation voltammograms of 25 ng/ml DNA at MFE, pH 7.7 under the experimental conditions as be cited in text: (a) blank; (b) 0; (c) 15; (d) 30; (e) 60; (f) 120; (g) 180; (h) 240 s and (i) 300 s.

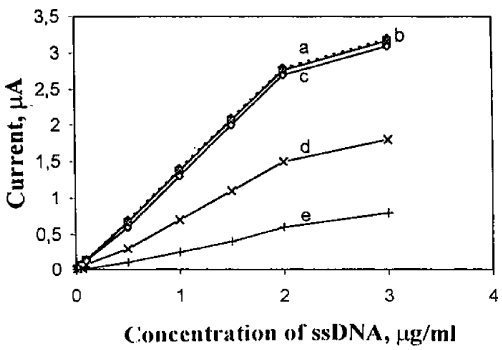


Fig. 10. Calibration plots of different ssDNA concentrations spikes from 5 ng/ml to 3 μg/ml at MFE applying 15 s accumulation time: (a) in absence of Tarabine PFS (dashed line), (b) in presence of 5×10^{-7} M Tarabine PFS, (c) 1×10^{-6} M Tarabine PFS, (d) 5×10^{-6} M Tarabine PFS and (e) 1×10^{-5} M Tarabine PFS, other parameters as cited in the text.

Table 2
Characteristic features of the calibration graphs for ssDNA at the MFE, after 15 s accumulation time, pH 7.7 in 0.1 M isotonic Sorensen buffer

Concentration range from 5 ng/ml to 3 μg/ml	Equation ($\mu A\ \mu g^{-1}\ ml^{-1}$)	Limit of linearity (μg/ml)	Standard deviation	Correlation coefficient
In absence of Tarabine PFS	$Y = 0.79X + 0.002$	2.0	1.0815	0.9988
In presence of 0.5 μM	$Y = 0.79X + 0.002$	2.0	1.0700	0.9984
In presence of 1.0 μM	$Y = 0.78X + 0.002$	2.0	1.0401	0.9982
In presence of 5.0 μM	$Y = 0.53X + 0.001$	2.0	0.5768	0.9895
In presence of 10.0 μM	$Y = 0.32X$	2.0	0.3036	0.9871

have been obtained after 15 s accumulation time in absence of Tarabine PFS (line a) and in presence of different concentrations of Tarabine PFS (lines b–e). It is observed that the presence of Tarabine PFS up to 1 μM has no effect on the determination of ssDNA molecules. All characteristic features of calibration graphs for different concentrations of ssDNA have been cited in Table 2. As we know, to have a significant analytical utility, the electrode response must exhibit not only high sensitivity but also well-defined and reproducible concentration dependence. For this purpose, the reproducibility of the adsorption process has been checked on 10 ng/ml ssDNA. Well-defined and reproducible peaks are obtained (by running nine experiments) having a mean value of the current of 1.45 μA with coefficient of variation 1.9%. The lowest concentration of DNA detected is 3 ng/ml at an accumulation time of 120 s. Therefore our procedure seems to be rapid, simple, sensitive and reproducible than those in previous reports [34,45].

3.6. Interferences and analytical utility for urine analysis

The effect of the following metal ions and the possible interfering organic substances, which are of great significance in biological matrices or can affect on the adsorption of drug on MFE surface, viz. Fe(III), Fe(II), Zn(II), Cd(II), Mn(II), Pb(II), Cu(II), ascorbic acid, uric acid, amino acids, gelatin and some surfactants (e.g. CTAB, SDS and Triton X-100) is tested at pH 7.7. It was found that Fe(II), Fe(III) and Pb(II) ions have not any effect on the adsorption of the drug. Zn(II), Mn(II) and Cd(II) ions have an effect when their concentrations exceed 50 times that of Tarabine PFS concentration. Cu(II) ion has an effect when its concentration reaches 0.51 μg/ml with appearing of a new peak at about −0.03 V, which is far enough about that of the drug under investigation. Addition of ascorbic acid and uric acid up to 1×0^{-4} and 1×10^{-3} M, respectively has no effect. The addition of gelatin and some surfactants (e.g. CTAB, SDS and Triton X-100) that can interfere with drug determination by co-adsorption onto MFE indicates that there is a slight depression in peak current if their concentration exceeds 12.5 mg/ml (about 25 times drug concentration). The influence of anions (e.g. SO_4^{2-}, NO_3^-, PO_4^{3-}, Cl^-) on the oxidation peaks of the investigated compound was studied. It was found that these anions have no any influence when their concentration exceeds 50 times of drug concentration (2×10^{-9} M).

Because of the high degree of adsorption, sensitivity and selectivity of the drug at MFE, the described method is suitable for the drug quantitation in biological fluids, viz. urine samples. The preparation of urine samples for drug determination is made as mention in previous work [46]. As reported that Tarabine PFS is degraded in the gastrointestinal tract. After oral administration, only about 20% of the drug reaches the circulation [7]. Peak concentrations of 2–50 μM are measurable in plasma after injection of 30–300 mg/l intravenously. Less than 10% of the injected dose is excreted unchanged in the urine within 12–24 h, while most appears as the inactive arabinosyl uracil (deaminated product). It is found that 8–10% of the injected drug dose was recovered in urine sample. Therefore, down to 0.23 ng/ml could be easily detected in urine samples.

4. Conclusion

This work has demonstrated that Tarabine PFS has two oxidation peaks and one reduction peak at

mercury film electrode. From the analytical point of view, the second oxidation peak could be easily used for the determination of nanomolar concentration levels of the drug in both aqueous and biological media. MFE gave rise to 4 and 25-fold enhancement of the adsorptive process than that at GCE and SMDE, respectively. Carbon paste electrode gave not any distinguished peaks for the drug. Also, our procedure was used for the adsorptive square-wave determination of nanolevels of ssDNA simply and reproducibility compared to other procedures with an explanation of the possible mechanism of interaction between ssDNA and Tarabine PFS at pH 7.7, due to MFE was behaving like human membrane in the living cell as negatively charged surface [47]. The proposed method is sensitive, giving excellent resolution of neighboring waves, reproducible and because of its simplicity and reliability is particularly suitable for routine analysis. Therefore, it allows for detecting down to 0.23 ng/ml Tarabine PFS in urine samples.

References

[1] A. Cringauz, Introduction to medical chemistry, Anticancer Drugs and their Mechanism of Action, Wiley, New York, 1997, p. 93.
[2] G.P. Bodey, E.J. Freireich, K.B. McCredie, V. Rodriquez, M.A. Burgress, E. Frei, Med. Pediatr. Oncol. 1 (1975) 29–35.
[3] J. Fuchs, J.G. Hengstler, D. Jung, G. Hiltl, J. Konietzko, F. Oesch, Mutat. Res. 342 (1995) 17–23.
[4] P.J.M. Sessink, M. Cerna, P. Rossner, A. Pastorkova, H. Bavarova, K. Frankova, R.B.M. Anzion, R.P. Bos, Mutat. Res. 309 (1994) 193–199.
[5] M.P. Demeo, S. Merono, A.D. Dabaille, A. Botta, M. Laget, H. Guiraud, G. Dumenil, Int. Arch. Occup. Environ. Health 66 (1995) 363–368.
[6] S. Roth, H. Norppa, H. Jarventaus, P. Kyyronen, M. Ahonen, J. Lehtomaki, H. Sainio, M. Sorsa, Mutat. Res. 325 (1994) 157–162.
[7] J.G. Hardman, A.G. Gilman, L.E. Limbird, Goodman and Gilman's The Pharmacological Basis of Therapeutics, ninth ed., McGraw-Hill, Washington, 1996.
[8] R.E. Notari, M.L. Chin, A. Cardoni, J. Pharm. Sci. 59 (1970) 27–32.
[9] D.H.W. Ho, Biochem. Pharmacol. 23 (1973) 1235–1244.
[10] D. Gningue, J.-J. Aaron, Talanta 32 (1985) 183–187.
[11] L.D. Kissinger, N.L. Stemm, J. Chromatogr. 353 (1986) 309–318.
[12] A.M. Ghe, G. Chiavari, J. Evgenidis, Talanta 33 (1986) 379–384.
[13] D. Dogrukol-Ak, M. Tuncel, Port. Electrochim. Acta 9 (1991) 331–338.
[14] M.S. Mahrous, M.M. Abdel-Khalek, H.G. Daabees, Y.A. Beltagy, Anal. Lett. 25 (1992) 1491–1501.
[15] J. Boutagy, D.J. Harvey, J. Chromatogr. A 156 (1978) 153–166.
[16] M.G. Pallavicini, J.A. Mazrimas, J. Chromatogr. 183 (1980) 449–458.
[17] H. Breithaupt, J. Schick, J. Chromatogr. 225 (1981) 99–106.
[18] M. Tuncel, R.E. Notari, L. Malspeis, J. Liq. Chromatogr. 4 (1981) 887–896.
[19] R.L. Schilsky, F.S. Ordway, J. Chromatogr. 337 (1985) 63–71.
[20] S.V. Galushko, I.P. Shishkina, A.T. Pilipenko, J. Chromatogr. 445 (1988) 59–70.
[21] V.D. Shatts, A.L. Brivkalne, Russ. J. Phys. Chem. (Engl. Transl.) 64 (1990) 1324–1328.
[22] S.V. Galushko, I.P. Shishkina, J. Pharm. Biomed. Anal. 10 (1992) 1093–1095.
[23] L. Krivankova, A. Kostalova, G. Vargas, J. Havel, P. Bocek, Electrophoresis 17 (1996) 1954–1958.
[24] R.L. Momparler, Med. Pediatr. Oncol. 10 (1982) 45–48.
[25] T. Sato, M. Morozumi, K. Kodama, A. Kuninaka, H. Yoshino, Cancer Treat. Rep. 68 (1984) 1357–1366.
[26] B.M. Mehta, M.B. Meyers, D.J. Hutchison, Cancer Chemother. Rep. 59 (1975) 515–522.
[27] R.T. Dorr, J.D. Liddil, B.S. Lyengar, A.F. List, W.A. Remers, Nucleosides Nucleotides 13 (1994) 1655–1668.
[28] J. Wang, Stripping Analysis: Principles, Instrumentation and Application, VCH Publishers, Deerfield Beach, Florida, 1985.
[29] T.M. Florence, J. Electroanal. Chem. 27 (1970) 273–281.
[30] D. Dogrukol-AK, M. Tuncel, Pharmazie 49 (1994) 928–929.
[31] D. Marin, C. Teijeiro, Bioelectrochem. Bioenerg. 28 (1992) 417–424.
[32] Y.M. Temerk, P. Valenta, H.W. Nurnberg, J. Electroanal. Chem. 100 (1979) 77–92.
[33] C. Teijeiro, D. Marin, J. Electroanal. Chem. 316 (1991) 119–131.
[34] J. Wang, S. Bollow, J.L.L. Paz, E. Sahlin, B. Mukherjee, Anal. Chem. 71 (1999) 1910–1913.
[35] D.D. Perrin, B. Dempsey, Buffers for pH and Metal Ion Control, Chapman & Hall, London, 1974.
[36] S.B. Adeloju, A. Hadjichari, Anal. Sci. 15 (1999) 95–100.
[37] R.K. Murray, D.K. Granner, P.A. Mayes, V.W. Rodwell, Harper's Biochemistry, Appleton & Lange, 23rd ed., California, 1993.
[38] M.R. Smyth, J.G. Osteryoung, Anal. Chem. 50 (1978) 1632–1637.
[39] E. Palecek, I. Postbieglova, J. Electroanal. Chem. 214 (1986) 359–371.
[40] N.A. El-Maali, J. Wang, Sens. Actuators B 76 (2001) 211–214.
[41] A.M. Oliveira Brett, S.H.P. Serrano, T.A. Macedo, D. Raimundo, M.H. Marques, M.A. La-Scalea, Electroanalysis 8 (1996) 992–995.
[42] P.T. Kissinger, W.R. Heineman, Laboratory Techniques in Electroanalytical Chemistry, Marcel Dekker, New York, 1984.
[43] W.H. Gmeiner, A. Skradis, R.T. Pon, J. Liu, Nucleic Acids Res. 26 (1998) 2359–2365.

[44] H.M. Geller, K.Y. Cheng, N.K. Goldsmith, A.A. Romero, A.L. Zhang, E.J. Morris, L. Grandison, J. Neurochem. 78 (2001) 265–275.
[45] F. Jelen, M. Tomschik, E. Palecek, J. Electroanal. Chem. 423 (1997) 141–148.
[46] N.A. El-Maali, Talanta 51 (2000) 957–968.
[47] E. Stutter, H. Schütz, H. Berg, Quantitative determination of cooperative anthracycline–dna binding, in: J. Lown (Ed.), Anthracycline and Anthracenedione-Based Anticancer Agents, Elsevier, Amsterdam, 1988, p. 245.