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Determination of the Antiretroviral Drug Zidovudine in Diluted Alkaline Electrolyte by Adsorptive Stripping Voltammetry at the Mercury Film Electrode

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Abstract

This paper describes a stripping method for the determination of zidovudine at the submicromolar concentration levels. This method is based on the controlled adsorptive accumulation of zidovudine at the thin-film mercury electrode, followed by a linear-sweep stripping voltammetry measurement of the surface species. Optimal experimental conditions include a NaOH solution of 2.0×10^{-3} mol·L^{-1} (supporting electrolyte), an accumulation potential of –0.30 V and a scan rate of 100 mV·s^{-1}. The response of zidovudine is linear over the concentration range 0.01 - 0.08 ppm. After an accumulation time of 5 minutes, the detection limit was found to be 0.67 ppb (2.5×10^{-9} mol·L^{-1}). More convenient methods to measure zidovudine concentration in the presence of the didanosine, acyclovir, nevirapine, lamivudine, and efavirenz, were also investigated. The presence of zidovudine together with ATP or ssDNA demonstrates the utility of this method.

Keywords: Zidovudine Determination, Antiviral Drugs, Ssdna, Thin-Film Mercury Electrode, Stripping Voltammetry

1. Introduction

Zidovudine (**Figure 1**) or azidothymidine (AZT) (also called ZDV), is a nucleoside analog reverse transcriptase inhibitor (NRTI), a type of antiretroviral drug. It is also sold under the names Retrovir and Retrovis, and as an ingredient in Combivir and Trizivir. It is an analog of thymidine. It was used as the first approved treatment for HIV. AZT does not destroy the HIV infection, but only delays the progression of the disease and the replication of virus, even at very high doses. During prolonged AZT treatment, HIV has the ability to increase its resistance to AZT by mutating its reverse transcriptase. In order to slow down the process of developing resistance, physicians generally recommend that AZT be given in combination with another reverse transcriptase inhibitor and an antiretroviral from another group, such as a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor [1-8].

Several methods have been discovered for the quantitative determination of zidovudine including chromatog-
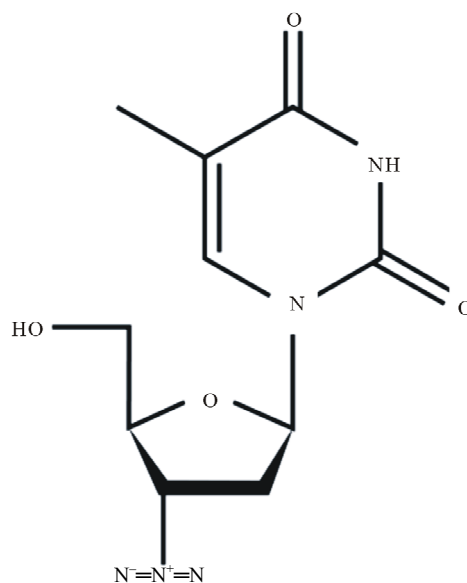

Figure 1. Structure of zidovudine (AZT); [1-[(2*R*,4*S*,5*S*)-4-azido-5-(hydroxymethyl)oxolan-2-yl]-5-methyl-1,2,3,4-tetra hydropyrimidine-2,4-dione].

raphy [9-15], fluorescence polarization immunoassay [16], using diamond paste based immunosensor [17], fluorescence spectroscopy [18] and voltammetry [19-24]. With the recent advancements in properties of the adsorptive stripping voltammetry, new methodologies have been developed for adenine, thymine, guanine, ATP, and DNA determinations employing alkaline solution with lower ionic strength as the supporting electrolyte [25-28]. Using this alkaline electrolyte, the present work found a new stripping voltammetric procedure to trace the detection of zidovudine based on its adsorption at the thin film mercury electrode. The advantages, instrumental parameters, and possible limitations of this procedure will be explained in this paper. Furthermore, the effects of a wide range of potentially interfering compounds such as didanosine, acyclovir, nevirapine, lamivudine, efavirenz, some metal ions, and ATP or ssDNA are examined.

2. Experimental

2.1. Apparatus

Linear-sweep stripping voltammograms were obtained with an EG&G PAR model 384-B Polarographic Analyser (Princeton Applied Research, Princeton, NJ, USA), equipped with an external cell and a Houston Ametek-DMP-40 series digital plotter. The working electrode was a glassy carbon electrode (GCE, 3.0 mm diameter, BAS-Bioanalytical Systems, Inc., West Lafayette, Indiana 47906, USA) containing thin-film mercury, an Ag/AgCl reference electrode with vicor tip and a platinum auxiliary electrode. A magnetic stirrer and stirring bar (Nalgene Cat. No. 6600 - 0010, Rochester, NY, USA) provided convective transport during the process of accumulation.

2.2. Forming Thin-Film Mercury Electrode

The thin mercury film was formed in a 10^{-2} mol·L^{-1} Hg(NO$_3$)$_2$ solution, prepared by the dissolving 0.4 g of mercury (II) nitrate into 100 mL of an acidified Milli-Q water (5% of HNO$_3$). A glassy carbon electrode (GCE, BAS) was first polished with alumina (PK-4, BAS) and then mounted with the help of a Teflon holder in a voltammetric cell provided with an Ag/AgCl reference electrode, a platinum auxiliary electrode, 1 mL of mercury (II) nitrate solution, 1 mL of 10^{-1} mol·L^{-1} potassium nitrate solution and 8 mL of purified water. The solution was purged with nitrogen for 240 s in order to eliminate the oxygen that was present initially. Mercury plating was carried over for 5 min at a cell of –0.9 V. After checking that the electrode was plated properly, the set of electrodes was rinsed with water and a new clean cell

containing the analyte solution was fitted.

2.3. Reagents

Water purified in a Milli-Q purification system (Millipore, Billerica, MA, USA) was used for all dilutions and sample preparations. All chemicals were of the analytical reagent grade. Zidovudine standard was used as it was received by the FarManguinhos – FIOCRUZ (Fundação Oswaldo Cruz – RJ). Stock solutions of 1000 ppm were prepared by dissolving 50 mg of the target reagent zidovudine into 5 mL of 2 mol·L^{-1} NaOH, 5 mL of ethylic alcohol and water until a volume of 50 mL was reached. Diluted zidovudine solutions of 100 or 10 ppm were prepared daily by dissolving 5 mL of 1000 or 100 ppm zidovudine into water until a volume of 50 mL was reached. Stock solutions of other HIV drugs were prepared using the same procedure described for zidovudine. The didanosine and aciclovir stock solutions were prepared without using along with ethylic alcohol. A 1000 ppm copper, other metal stock solutions (atomic absorption standard solution, Sigma-Aldrich Brasil Ltda.), were used and diluted as required for standard additions. Stock solutions of 1000 ppm of adenosine 5'-tripho- sphate and disodium salt hydrate (ATP) were prepared by dissolving 10 mg of the target reagent into 2 mL of diluted perchloric acid (10^{-1} mol·L^{-1}). The subsequent solution was heated at 70°C for 30 seconds. After being heated, the sample was cooled down and diluted with water to a volume of 10 mL. Single-stranded calf thymus DNA (Cat. No. D-8899; Lot 43H67951) was used as it was received from Sigma. A 500 μg DNA/mL stock solution (around 5 mg/10 mL; lyophilized powder containing 63% DNA) was prepared according to the ATP procedure. The final solution was stored at 4°C.

2.4. Procedure

A known volume (10 mL) of the supporting electrolyte solution (2.0×10^{-3} mol·L^{-1} sodium hydroxide (with 1% v/v of ethylic alcohol)) was added to the voltammetric cell and degassed with nitrogen for 8 min (and for 60 seconds before each adsorptive stripping cycle). First, the condition potential (usually –0.9 V) was applied to the electrode for a set amount of time (usually 60 s). Afterwards, the initial potential (–0.30 V) was applied to the electrode for a set amount of time (usually 90 s). The solution was stirred slowly throughout this time. The stirring was then stopped, and after 30 s, the voltammogram was recorded by applying a negative-going potential scan. The scan (at 100 mV·s^{-1}) was stopped at –1.10 V, and the adsorptive stripping cycle was repeated using the same thin-film mercury. After the background stripping volt-

ammograms were obtained, aliquots of the zidovudine standards were introduced. The entire procedure was automated, as controlled by 384-B Polarographic Analyser. Throughout this operation, nitrogen was passed over the surface of the solution. All data were obtained at room temperature (25˚C).

3. Results and Discussion

3.1. Parameters Affecting the Adsorptive Stripping Behavior

Figure 2 compares the differential-pulse, linear-scan and linear-sweep adsorptive stripping voltammograms using 0.10 ppm zidovudine in a 2.0×10^{-3} mol·L^{-1} NaOH solution (with 1% v/v of ethylic alcohol) after 90 seconds of preconcentration, stirring at –0.30 V. A mercury film was used as a work electrode. After an equilibrium time of 30 s, the differential pulse (A) and linear (B) or cyclic cathodic voltammogram (C) was recorded at 50 (A) and 100 (B,C) mV·s^{-1}, respectively. Both scan modes offer excellent signal-to-background characteristics. Linear scans, however, offer a higher current peak and a greater speed, and are recommended for the determination of zi-

dovudine. The linear-sweep stripping mode yields a well- defined peak, with half-width ($b\frac{1}{2}$) of 80 mV. The zidovudine cyclic cathodic peak (I_p) appears at –0.68V (E_p). No anodic peak was observed in the first scan.

Figure 3 shows the linear-sweep adsorptive voltammogram for 0.1 ppm of zidovudine in a 2.0×10^{-3} mol·L^{-1} NaOH solution (with 1% v/v of ethylic alcohol), using a pre-concentration time of 60 s, while being stirred however, at –0.15 (A) and –0.30 (B) V. After an equilibrium time of 30 s, the linear-sweep voltammogram was recorded at 100 mV·s^{-1}. With accumulation potential at –0.15 V, the zidovudine cyclic voltammogram showed two peaks at –0.28 and –0.62 V. Only a single, well- defined, higher cathodic peak (I_p) with half-width ($b\frac{1}{2}$) of 80 mV peak appears at –0.68 V(E_p). This occurs when the accumulation potential used was of –0.30 V. This accumulation potential was used throughout this study.

Other chemicals and instrumental parameters such as the supporting electrolyte, the pH level, the accumulation time, and the scan rate (which directly affects the zidovudine adsorptive stripping peak response) were also optimized. The adsorption properties of the zidovudine vary depending on the composition of the supporting electrolyte. Various electrolytes, e.g. Briton-Robinson,
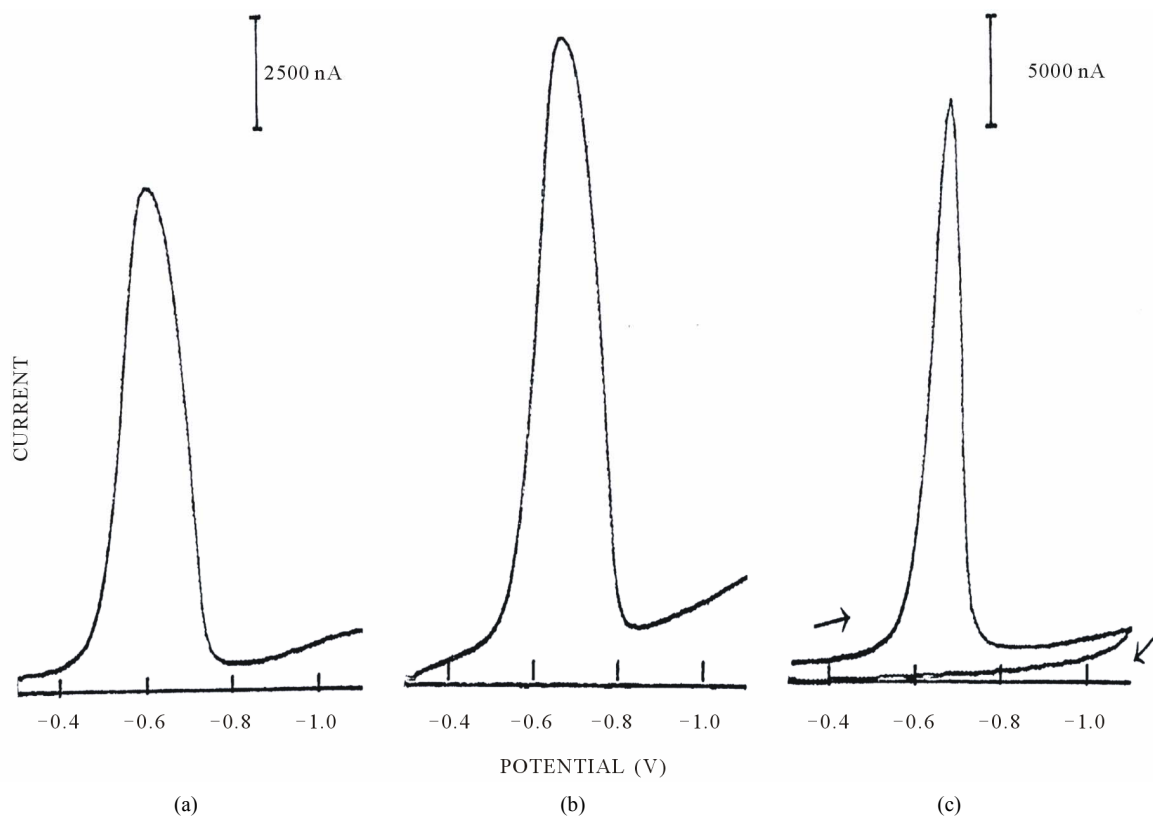

Figure 2. Differential-pulse (a), linear-scan (b) and linear-sweep adsorptive (c) voltammograms of 0.10 ppm zidovudine in 2.0 × 10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol). Condition time, 60 s at –0.90 V; accumulation time, 90 s at –0.30 V with stirring; amplitude pulse, 50 mV (a); scan rate, 50(a) and 100(b,c) mV·s^{-1}; thin-film mercury electrode (5 min at –0.9 V).
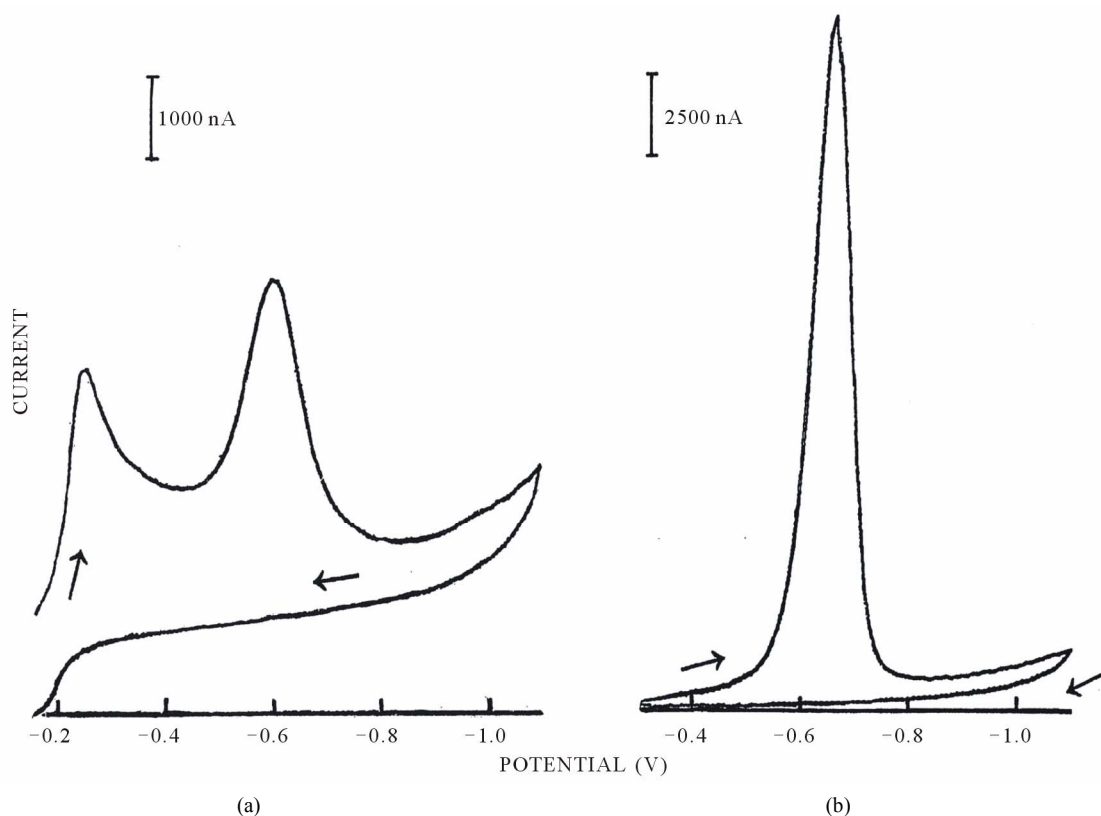
Figure 3. Linear-sweep adsorptive voltammogram of a 0.10 ppm zidovudine solution in 2.0 × 10⁻³ mol·L⁻¹ NaOH (with 1% v/v of ethylic alcohol). Condition time, 60 s at –0.90 V; accumulation time, 60 s at –0.15 (a) and –0.30 (b) V with stirring; scan rate, 100 mV·s⁻¹; thin-film mercury electrode (5 min at –0.9 V).

phosphate and NaOH solution, were determined to be suitable media for the adsorptive stripping measurement of zidovudine. The best results (with respect to signal enhancement and reproducibility) were obtained using the NaOH electrolyte. The alkaline medium was employed throughout this study. The adsorptive stripping signal of zidovudine depends on the sample pH.

Figure 4 shows the dependence of the zidovudine peak current on the solution's pH (from 2 to 11). No response to zidovudine was observed in solutions more acidic than pH 6. Increasing the pH level from 6.5 to 11 resulted in rapid increase in the zidovudine peak current. However, the stability of the zidovudine peak in aqueous solutions decreased when the pH was above 11. Because of this, a pH of approximately 11 was used to satisfy the sensitivity and stability requirements throughout the experiment.

Figure 5 shows a detailed study of the effect of the accumulation potential (from +0.05 to –0.30 V) on the stripping zidovudine voltammograms. Parameters like the potential peak (E_p), the half-width ($b_{1/2}$) and the current peak (I_p) were also observed. The first zidovudine peak at approximately 0.28 V is closer than the other HIV drugs' peaks, which were previously analyzed as

possible interferences to ziduvudine determination. The second peak, at –0.68 V, is in agreement with the peak observed in **Figure 3(b)**, which shows a very welled-fined peak with best half-width/ background resolution. The accumulation potential at –0.30 V was then confirmed as ideal and used throughout this study.

Figure 6 shows a study of the effect of the scan rate at 10 (a) and 200 mV·s⁻¹ (b) on the zidovudine stripping voltammograms. With a scan rate of 200 mV·s⁻¹, the peak current for a 0.10 ppm zidovudine solution was about 30.5 times larger than the corresponding peak obtained with 10 mV·s⁻¹ response. However, this gain in sensitivevity is accompanied by broadening peaks. The peak current (I_p) for the surface-adsorbed zidovudine is directly proportional to the scan rate (v). A plot of I_p vs v was linear (correlation coefficient, 0.998), with a slope of 235.3, over the 10 - 100 mV·s⁻¹ range. Overall, a scan rate of 100 mV·s⁻¹ would be the best solution when considering the sensitivity, resolution and speed requirements.

Figure 7 shows the dependence of the linear cyclic current peak along with the pre-concentration time. The peak increases linearly with time and then levels off. Such time-dependent profiles represent the corresponding
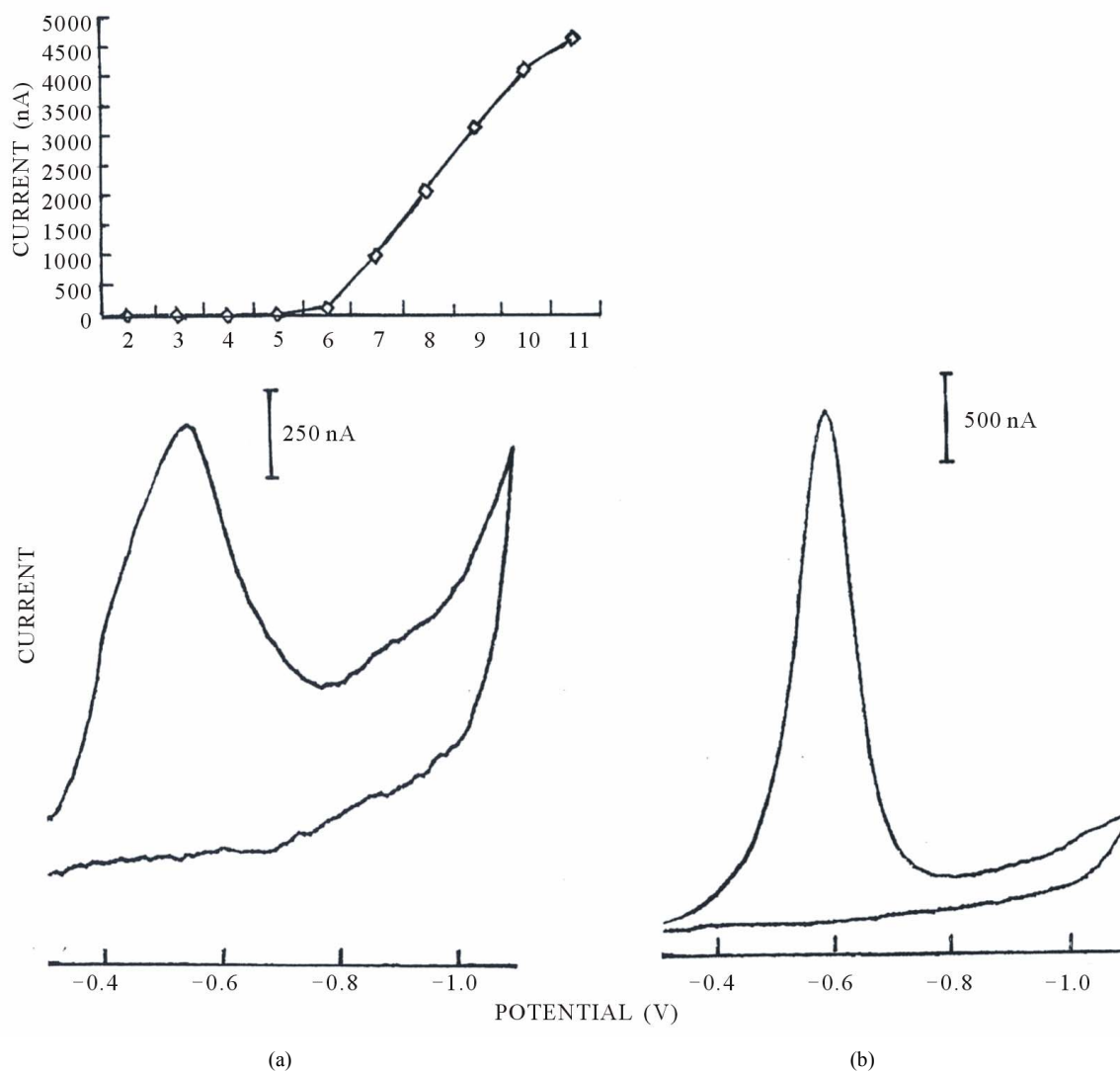
Figure 4. Effect of pH (A-7; B-11) on the linear-sweep adsorptive stripping voltammograms of 0.05 ppm zidovudine. Conditions: time, 60 s at –0.90 V; accumulation time, 90 s at –0.30 V; scan rate, 100 mV·s^{-1}; equilibrium time, 30 s; thin-film mercury electrode (5 min at –0.9 V). Also shown is the resulting current peak versus pH plot.

absorption isotherms since the peak current depends on the amount adsorbed. With 90 s of pre-concentration, the peak current for a 0.10 ppm zidovudine solution was about 13.3 times larger than the corresponding peak obtained with a direct (0 s) response. The resulting plot of peak current vs. accumulation time (0 - 60 s) is linear (slope 240.8 nA·s^{-1} and correlation coefficient, 0.993).

3.2. Quantitative Utility

The effect of the pre-concentration associated with the adsorption process yields a significantly lower detection limit compared to the corresponding solution measurements. A detection limit of 0.67 ppb (2.5×10^{-9} mol·L^{-1}) was estimated from quantifying 0.01 ppm after a 5-min

accumulation (S/N = 2). Thus, 6.7 ng was detected in the 10 mL of solution used. The reproducibility was estimated by was taking ten successive measurements of a stirred 0.04 ppm zidovudine solution (other conditions: supporting electrolyte, 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol); condition time, 60 s at –0.9 V; accumulation time, 90 s at –0.3 V; final potential, –1.1 V; scan rate, 100 mV·s^{-1}; equilibrium time, 30 s and thin-film mercury electrode). The mean peak current was 5503 nA with a range of 5407 - 5593 nA and a relative standard deviation. The E_p and the $b_{1/2}$ remained the same at –0.68 V and 80 mV, respectively. **Figure 8** displays voltammograms for increasing zidovudine concentration (A –0.02 and B –0.08 ppm) after 90 s of accumulation. Well-defined stripping peaks (at –0.68 V) were
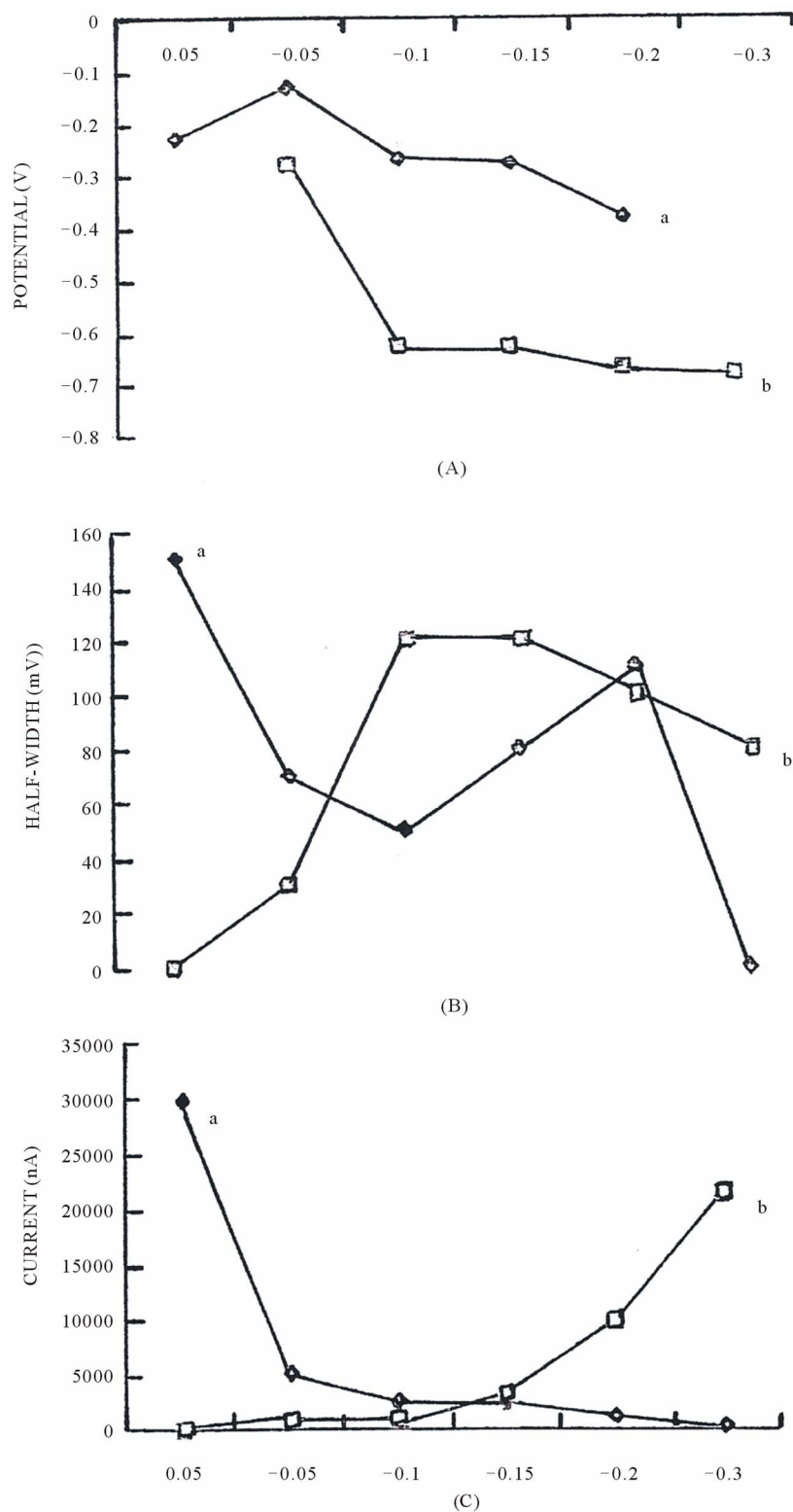
Figure 5. Effect of accumulation potential on the linear-sweep adsorptive stripping voltammograms of 0.10 ppm zidovudine solution in $2.0 \times 10^{-3} \cdot mol \cdot L^{-1}$ NaOH (with 1% v/v of ethylic alcohol). Conditions: time, 60 s at –0.90 V; accumulation time, 60; scan rate, 100 mV·s^{-1}; equilibrium time, 30 s; thin-film mercury electrode (5 min at –0.9 V). (A) Accumulation potential versus potential zidovudine peak, (B) versus half-width of zidovudine peak and (C) versus current zidovudine peak. (a) Zidovudine first peak and (b) second zidovudine peak.
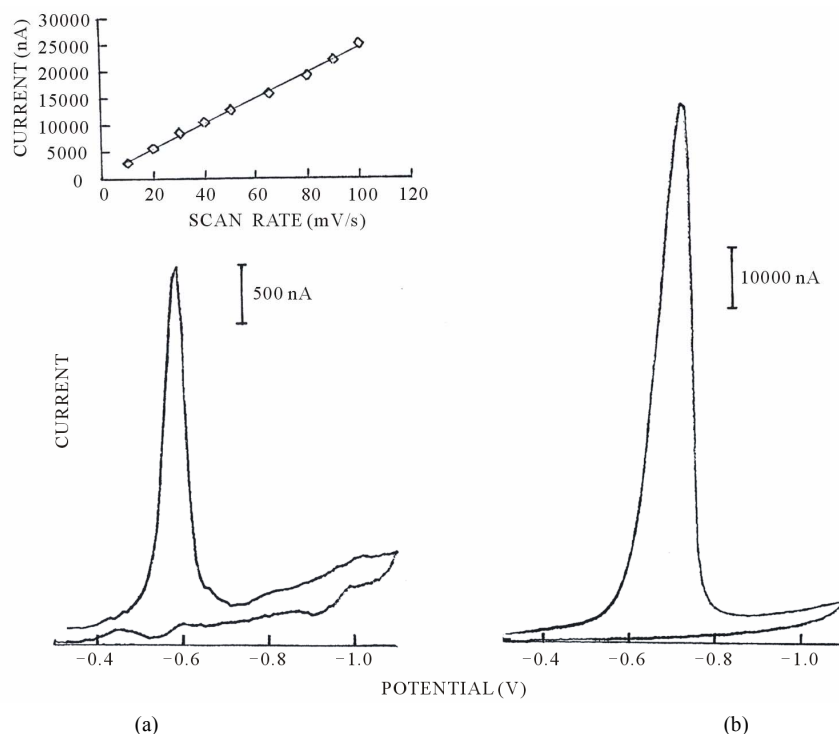
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Figure 6. Effect of scan rate on the linear-sweep voltammograms of 0.10 ppm zidovudine in 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol). Accumulation time, 90 s at –0.30 V. Final potential, –1.1 V. The (a) and (b) curves are relative to scan rate of 10 and 200 mV·s^{-1}, respectively. Conditions: time, 60 s at –0.90 V; equilibrium time, 30 s; thin-film mercury electrode (5 min at –0.9 V). Also shown is the resulting current peak versus scan rate plot.
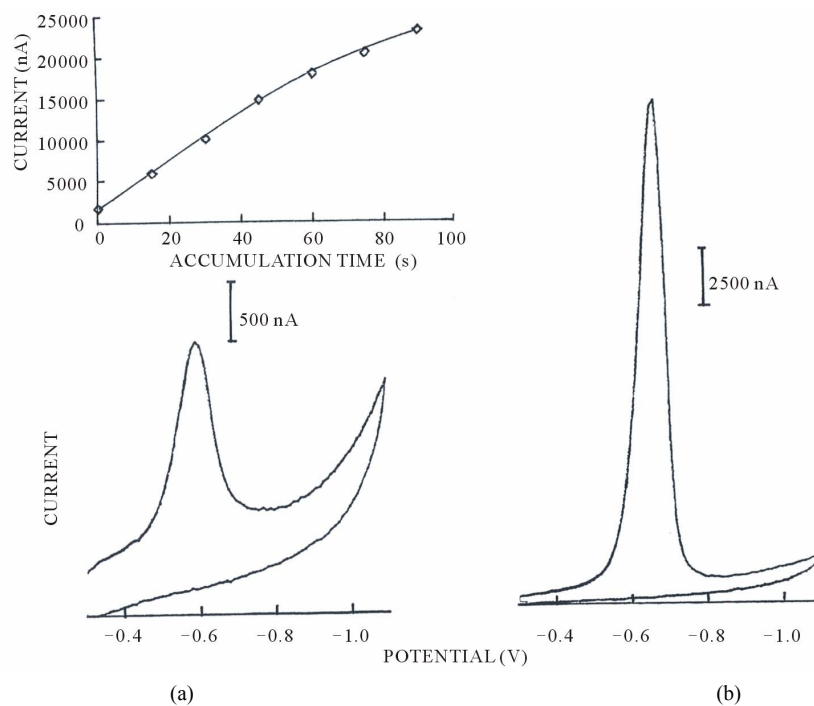

Figure 7. Effects of accumulation time at –0.30V on the linear-sweep adsorptive stripping voltammograms of 0.10 ppm of zidovudine in 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol). The (a) and (b) curves are relative to accumulation times of 5 and 90 s, respectively. Condition time, 60s at –0.90 V; scan rate, 100 mV·s^{-1}, equilibrium time, 30 s; thin-film mercury electrode (5 min at –0.9 V). Also shown is the resulting current peak versus accumulation time plot.
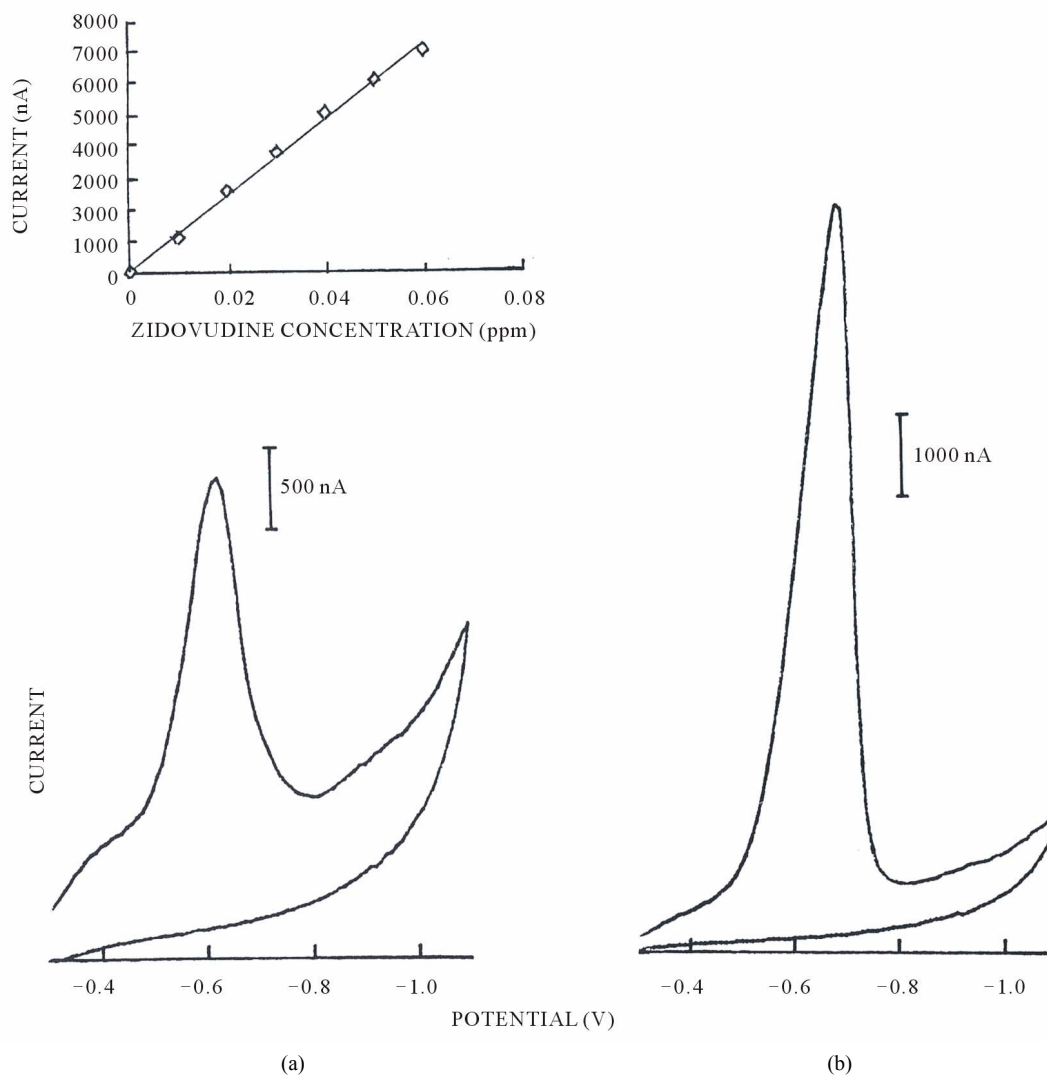
Figure 8. Linear-sweep adsorptive stripping voltammograms obtained after increasing the zidovudine concentration in a solution of 2.0 × 10⁻³ mol·L⁻¹ NaOH(with 1% v/v of ethylic alcohol). The (a) and (b) curves are relative to 0.02 and 0.08 ppm of zidovudine concentration, respectively. Accumulation time, 90 s at –0.30 V. Condition time, 60 s at –0.9 V. Scan rate, 100 mV·s⁻¹. Final potential at –1.1 V. Equilibrium time, 30 s. Thin-film mercury electrode (5 min at –0.9 V). Also shown is the resulting calibration curve plot.

observed between the 0.01 and the 0.06 zidovudine concentration ranges. The plot of peak current versus concentration that resulted is linear (slope 119014 nA/ppm; correlation coefficient, 0.998). This linearity prevails as long as linear isotherm conditions (low surface coverage) continue to exist. **Table 1** shows a summary of the optimized conditions for zidovudine determination by linear cyclic adsorptive stripping voltammetry at the mercury film electrode.

Practical applications of the linear-sweep adsorptive stripping analyses may be interfered with by the presence of metal ions and/or surface active compounds. With respect to the surface reaction, double layer changes or direct interactions deriving from these substances may inhibit or aid in the accumulation of the analyte. Measurements of 0.05 ppm zidovudine (other conditions: supporting electrolyte, 2.0 × 10⁻³ mol·L⁻¹ NaOH (with 1% v/v of ethylic alcohol) ; condition time, 60s at –0.9 V; accumulation time, 90 s at –0.30 V; final potential, –1.1 V; scan rate, 100 mV·s⁻¹; equilibrium time, 30 s and thin-film mercury electrode) were not affected by the addition of up to 0.03 ppm of lead (II); up to 0.05 ppm of cobalt (II); up to 0.06 ppm of nickel (II) and up to 0.10 ppm of iron (III) or copper (II) or cadmium (II) or zinc (II). Preliminary studies were developed for the detemination of zidovudine in the presence of other antiretroviral drugs for the treatment of human immunodeficiency virus (HIV): didanosine, acyclovir, nevirapine

Table 1. Optimized conditions for zidovudine determination by linear cyclic adsorptive stripping voltammetry at the mercury film electrode.

Parameters	Optimized conditions
Thin-film mercury electrode	5 minutes at –0.9 V
Zidovudine concentration range	0.01 - 0.08 ppm
Electrolyte	2.0×10^{-3} mol·L^{-1} NaOH
Scan mode	Linear cyclic
Initial potential	–0.3V
Final potential	–1.1 V
Scan rate	100 mV·s^{-1}
Condition potential	–0.9 V
Condition time	60 seconds
Accumulation potential	–0.3 V
Accumulation time	90 seconds
Equilibrium time	30 seconds

and lamivudine. Measurements of 0.05 ppm zidovudine (other conditions: supporting electrolyte, 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol); condition time, 60s at –0.9 V; accumulation time, 90 s at –0.30 V; final potential, –1.1 V; scan rate, 100 mV·s^{-1}; equilibrium time, 30 s and thin-film mercury electrode) were not affected by the addition of up to 0.06 ppm of lamivudine or up to 0.10 ppm of acyclovir, nevirapine, didanosine or efavirenz.

Figure 9 illustrates the method's suitability for the determination of zidovudine through linear-sweep adsorptive stripping voltammetry in a synthetic sample containing several antiretroviral drugs (efavirenz, nevirapine, didanosine, lamivudine, zidovudine and acyclovir; all with 2.0 ppm of concentration). Four successive standard additions to the sample resulted in well-shaped adsorptive stripping peaks. The zidovudine peak in the original sample (curve A) can, therefore, be quantified
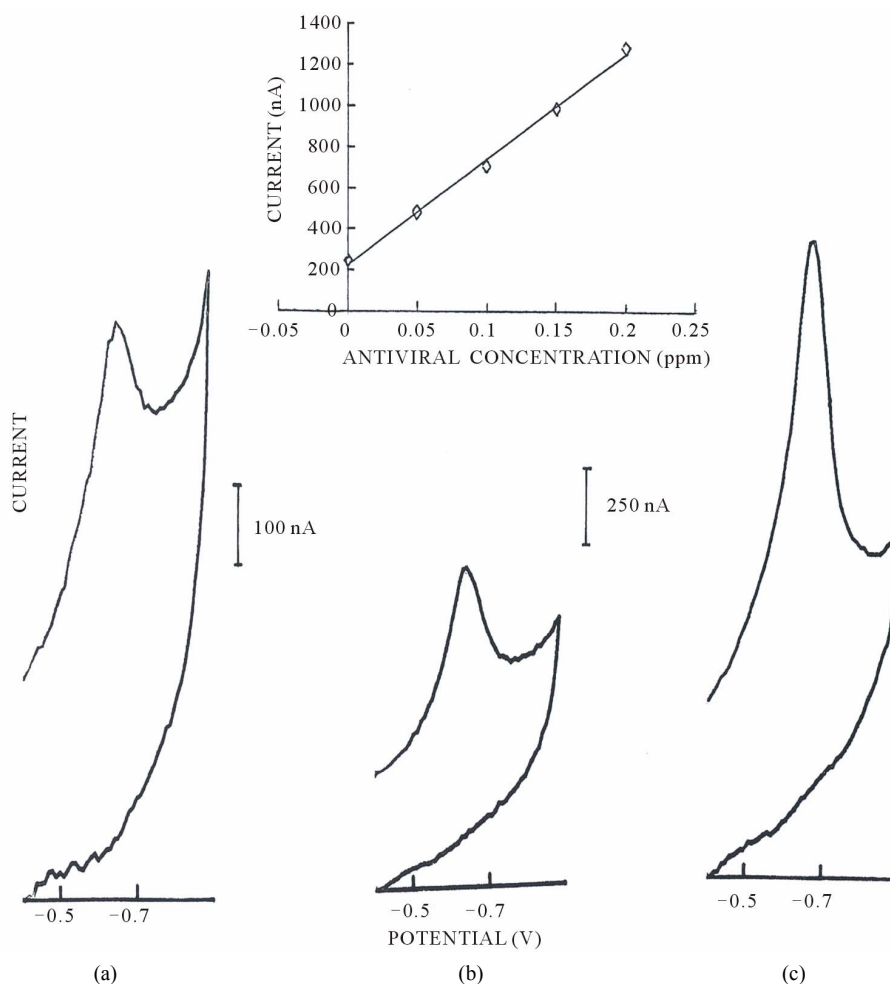

Figure 9. Illustration of zidovudine determination in a synthetic sample contain several antiretroviral drugs (efavirenz, nevirapine, didanosine, lamivudine, zidovudine and acyclovir; all with 2 ppm of concentration) by linear-sweep adsorptive stripping voltammetry. Supporting electrolyte, 10 mL of 2.0×10^{-3} mol·L^{-1} NaOH. (a) addition of 150 µL of synthetic sample; (b) (0.05 ppm) and (c) (0.20 ppm) addition of standard zidovudine. Condition time, 60s at –0.9 V. Accumulation time, 90 s at –0.40 V. Potential final, –0.9 V. Scan rate, 100 mV·s^{-1}. Equilibrium time, 30 s. Thin-film mercury electrode (5 min at –0.9 V).

based on the resulting standard addition plot (also show in **Figure 9**). Because of the inherent sensitivity of this method, short (90 s) accumulation times should be used. Five consecutive analyses of the sample yielded an average value of 2.7 ppm with a standard deviation of 0.5 ppm, in "relative" agreement with the zidovudine value (2.0 ppm). Preliminary studies were also developed for the determination of zidovudine in the presence of ssDNA and ATP. The current measurements of 0.05 ppm of zidovudine (other conditions: supporting electrolyte, 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol); condition time, 60 s at –0.9 V; accumulation time, 90 s at –0.30 V; final potential, –1.1 V; scan rate, 100 mV·s^{-1}; equilibrium time, 30 s and thin-film mercury electrode) were not affected by the addition of up to 0.10 ppm of ATP or 0.06 ppm of ssDNA.

The **Figure 10** shows the linear cyclic adsorptive stripping voltammograms of 0.05 ppm zidovudine in the presence of copper II ions and ssDNA (0.10 ppm) using an accumulation time of 90 s at –0.15 (A) and –0.30 (B) V (other conditions: supporting electrolyte, 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol); condition time, 60 s at –0.9 V; final potential, –1.2 V; scan rate, 100 mV·s^{-1}; equilibrium time, 30 s and thin-film mercury electrode). When the accumulation potential was applied to the –0.15 V (prior to the scan) the zidovudine peak appears at –0.35 V and the ssDNA peak at –0.78 V, but at –0.30 V the ziduvudine peak appears at –0.63 V and the ssDNA at –0.79 V. Without the present copper II ions, the ssDNA current peak does not appear efficiently. The zidovudine and ssDNA current peaks increase with higher accumulation times. A small peak of the copper (II) ion was also observed in the anodic scan at –0.40 V.

4. Conclusions

This paper has thoroughly described an effective means for the determination of trace levels of zidovudine. The use of the simple and diluted alkaline electrolytes provided a sensitive and selective adsorptive stripping voltammetric method for zidovudine determination. This approach is rapid, yields a high sensitivity, and is largely unaffected by a large number of commonly presented antiretroviral drugs for the treatment of human immunodeficiency virus (HIV), such as didanosine, acyclovir, nevirapine and lamivudine. The zidovudine peak is separated by 0.43 V with the ssDNA stripping peaks. In particular, this approach offers similar efficiency in comparison to electrochemistry [24] and chromatographic methods. Additionally, further studies using diluted alkaline solution as the supporting electrolyte and film mercury electrode modified *in situ* by metallic ions can be
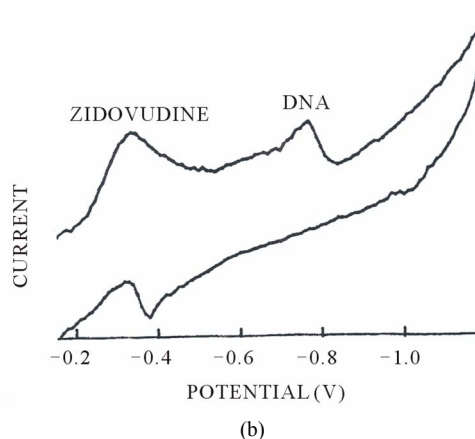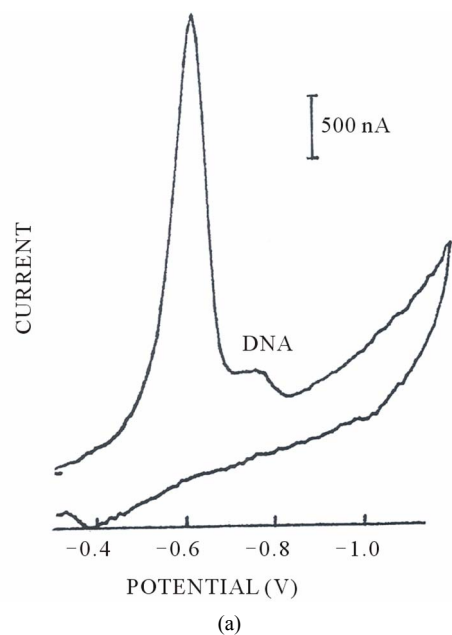

(a)

(b)

Figure 10. Linear-sweep adsorptive stripping voltammogram of zidovudine (0.05 ppm) in the presence of copper II ions and DNA (0.10 ppm) in a solution of 2.0×10^{-3} mol·L^{-1} NaOH (with 1% v/v of ethylic alcohol). Accumulation time, 90 s at –0.15 (a) and –0.30 (b) V. Condition time, 60 s at –0.9 V. Scan rate, 100 mV·s^{-1}. Final potential at –1.2 V. Equilibrium time, 30 s. Thin-film mercury electrode (5 min at –0.9 V).

used to detect other drugs and DNA-intercalating dyes, as well as amino-acids, peptides and protein determinations.

5. Acknowledgements

6. References

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