



Open Access : : ISSN 1847-9286

www.jESE-online.org

Original scientific paper

Didanosine determination in diluted alkaline electrolyte by adsorptive stripping voltammetry at the mercury film electrode

PERCIO AUGUSTO MARDINI FARIAS[✉], ARNALDO AGUIAR CASTRO*, ANA ISA PEREZ CORDOVES*

Department of Chemistry, Pontifícia Universidade Católica do Rio de Janeiro, 22451-900, Rio de Janeiro, RJ, Brazil

*Fac. Química, Química Analítica, Universidad de La Habana, Cuba

[✉]Corresponding Author: E-mail: pfarias@puc-rio.br; Tel.: ++55 21 35271317

Received: March 27, 2012; Published: August 30, 2012

Abstract

A stripping method for the determination of didanosine at the submicromolar concentration levels is described. The method is based on controlled adsorptive accumulation of didanosine at thin-film mercury electrode followed by a linear cyclic scan voltammetry measurement of the surface species. Optimum experimental conditions include a NaOH solution of 2.0×10^{-3} mol L⁻¹ (supporting electrolyte), an accumulation potential of -0.20 V, and a scan rate of 100 mV s⁻¹. The response of didanosine is linear over the concentration range 0.01 – 0.10 ppm. For an accumulation time of 7 minutes, the detection limit was found to be 0.43 ppb (1.0×10^{-9} mol L⁻¹). The more convenient relation to measuring the didanosine in presence of the metals ions, efavirenz, acyclovir, nevirapine, indinavir, nelfinavir, saquinavir, lamivudine and zidovudine were also investigated. The utility of the method is demonstrated by the presence of didanosine together with hypoxanthine, ATP or DNA

Keywords

Didanosine determination; metals ions; antiretroviral drugs; hypoxanthine; ATP; DNA; thin-film mercury electrode; linear cyclic scan stripping voltammetry.

Introduction

Didanosine (Figure 1) is a nucleoside analogue of adenosine. It differs from other nucleoside analogues, because it does not have any of the regular bases, instead it has hypoxanthine attached to the sugar ring. Within the cell, didanosine is phosphorylated to the active metabolite of dideoxyadenosine triphosphate (ddATP) by cellular enzymes. Like other anti-HIV nucleoside

analogs, it acts as a chain terminator by incorporation and inhibits viral reverse transcriptase by competing with natural dATP. [1-3].

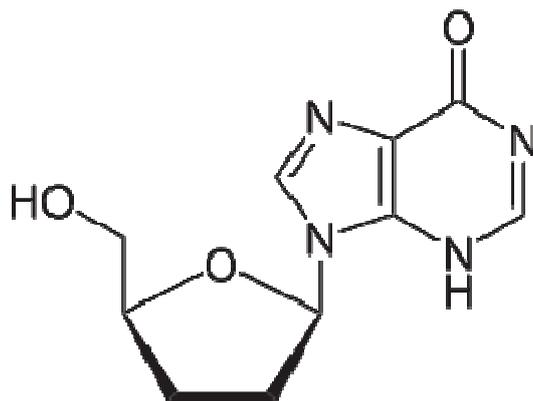


Figure 1. Didanosine; [9-[(2R,5S)-5-(hydroxymethyl)oxolan-2-yl]-6,9-dihydro-3H-purin-6-one]

Several chromatographic [4-11] analytical methods have been developed for the determination of didanosine but only one electroanalytical work was reported [12]. With the recent advances in capabilities of the adsorptive stripping voltammetry, new methodologies have been developed for adenine, thymine, guanine, ATP, DNA and antiretroviral drugs using alkaline solution with low ionic strength as the supporting electrolyte [13-20]. The present work reports a new stripping voltammetric procedure for trace detection of didanosine based on its adsorption at the thin film mercury electrode. The advantages, instrumental parameters, and possible limitations of this procedure are also explained in this paper. Furthermore, the effects of a wide range of potentially interfering compounds such as efavirenz, acyclovir, nevirapine, indinavir, nelfinavir, saquinavir, lamivudine, zidovudine, some metal ions, hypoxanthine, ATP and DNA were examined.

Experimental

Linear cyclic 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) with thin-film mercury, an Ag/AgCl reference electrode with vycor 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 accumulation step.

Stock solutions of 10^{-2} mol L⁻¹ Hg(NO₃)₂ were initially prepared by dissolution of 0.4 g of mercury (II) nitrate in 100 mL of an acidified Milli-Q water (5 % of HNO₃). The thin mercury film was formed using a glassy carbon electrode (GCE, BAS) initially 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, and containing 1 mL of stock 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 to eliminate the oxygen initially present. 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. Each thin mercury film formed on glassy carbon electrode is stable during two hours [18-20].

Water purified in a Milli-Q purification system (Millipore, Billerica, MA, USA) was used for all dilutions and sample preparations. All chemicals were analytical reagent grade. Didanosine

standard was used as received by the Instituto Nacional de Controle de Qualidade em Saúde - Brazil (INCQS – lot L1, 100.2%). Stock solutions of 1000 ppm were prepared dissolving 50 mg of the target reagent didanosine into 5 mL of 2 mol L⁻¹ NaOH and water until a volume of 50 mL was reached. Diluted didanosine solutions of 100 or 10 ppm were prepared daily using 5 mL of 1000 or 100 ppm didanosine into water until 50 mL was reached. Stock solutions of acyclovir were prepared in accordance with didanosine procedure. Stock solutions of 1000 ppm of other HIV drugs were prepared dissolving 50 mg of the target reagent into 5 mL of 2 mol L⁻¹ NaOH, 5 mL of ethylic alcohol and water until a volume of 50 mL was reached. A 1000 ppm copper and other metals ions stock solutions (atomic absorption standard solution, Sigma-Aldrich Brasil Ltda.) were used, and diluted as required for standard additions. Sigma Chemicals (Sigma-Aldrich Brasil Ltda., São Paulo-SP, Brasil) stock solutions of 1000 ppm were prepared by dissolving 25 mg of the target reagent hypoxanthine plus solid NaOH with 25 mL of water (to achieve a final concentration of 0.1 mol L⁻¹ NaOH). Stock solutions of 1000 ppm of adenosine 5'-triphosphate, disodium salt hydrate (ATP) was prepared by dissolving 10 mg of the target reagent in 2 mL of diluted perchloric acid (10⁻¹ mol L⁻¹) with an subsequent solution heating at 70 °C during 30 seconds. After heating, the sample was cooled down, and diluted to 10 mL with water. Single-stranded calf thymus DNA (Cat. No. D-8899; Lot 43H67951) was used as received from Sigma. A 500 µg DNA mL⁻¹ stock solution (around 5 mg 10 mL⁻¹; Lyophilized powder containing 63% DNA) was prepared according to ATP procedure. The solutions were stored in the dark at 4 °C.

A known volume (10 mL) of the supporting electrolyte solution (2.0×10⁻³ mol L⁻¹ sodium hydroxide) was added to the voltammetric cell and degassed with nitrogen for 8 min (and for 60 seconds before each adsorptive stripping cycle). Initially, the condition potential (usually -0.9 V) was applied to the electrode for a selected time (usually 60 s). The conditioning potential is used for cleaning the surface of the thin mercury electrode. Afterwards the initial potential (usually -0.20 V) was applied to the electrode with a selected time (usually 240 s), while the solution was slowly stirred. The stirring was then stopped, and after 30 s, the voltammogram was recorded by applying a negative-going potential scan. The scan (usually at 100 mV s⁻¹) was terminated at -0.90 V, and the adsorptive stripping cycle was repeated with the same thin-film mercury. After the background stripping voltammograms had been obtained, aliquots of the didanosine standards were introduced. The entire procedure was automated, as controlled by 384-B Polarographic Analyser. Throughout this operation, nitrogen was passed over the solution surface. All data were obtained at room temperature (25 °C).

Results and discussion

Figure 2 compares differential-pulse, linear-scan and linear cyclic adsorptive stripping voltammograms for 0.05 ppm didanosine in a 2.0×10⁻³ mol L⁻¹ NaOH solution, after 120 seconds preconcentration with stirring at -0.20 V. A mercury film was use as work electrode. After equilibrium time of 30 s the differential pulse (A), linear (B) or cyclic cathodic voltammogram (C) were recorded at 25 (A) or 100 (B,C) mV s⁻¹. Both scan modes offer excellent signal-to-background characteristics but linear scan offers higher current peak and greater speed, and is recommended for the determination of didanosine. The linear cyclic stripping mode yield well-defined peak, with half-width (b½) of 130 mV, and was used throughout the further study. The didanosine cyclic cathodic peak of 2407 nA (*I_p*) appears at -0.56V (*E_p*). No anodic peaks were observed in the first scan.

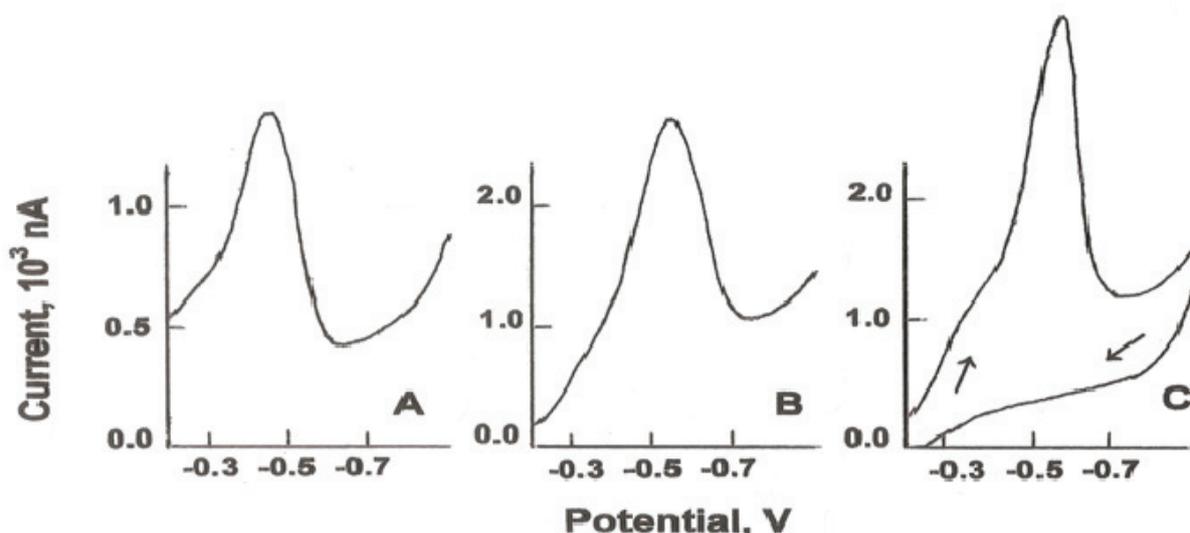


Figure 2. Differential-pulse (A), linear-scan (B) and adsorptive cyclic (C) voltammograms of 0.05 ppm didanosine in $2.0 \times 10^{-3} \text{ mol L}^{-1}$ NaOH. Conditioning time, 60 s at -0.90 V; accumulation time, 120 s at -0.20 V with stirring; amplitude pulse, 50 mV (A); scan rate, 25 (A) and 100 (B,C) mV s^{-1} ; thin-film mercury electrode

Several other chemical and instrumental parameters such as supporting electrolyte, pH, accumulation potential and time, and scan rate, which directly affect the didanosine adsorptive stripping peak response, were optimized. The adsorption properties of the didanosine can vary with the composition of the supporting electrolyte. Various electrolytes, *e.g.* Britton-Robinson buffer, phosphate buffer and NaOH solution, were evaluated as suitable media for the adsorptive stripping measurement of didanosine. Best results (with respect to signal enhancement and reproducibility) were obtained in NaOH electrolyte. This alkaline medium was employed throughout this study.

The adsorptive stripping signal of didanosine depends on the sample pH. Figure 3A, shows the dependence of the didanosine peak current on the solution pH (from 2 to 12). No response to didanosine was observed in solutions more acidic than pH 7. Increasing pH from 8 to 11 resulted in a rapid increase in the didanosine peak current. However, the stability of didanosine peak in aqueous solutions decreases with pH above of 11. Accordingly, the solution of approximately pH 11 was used throughout to satisfy the sensitivity and stability requirements.

The effect of accumulation potential was observed from +0.05 to -0.40 V and the highest didanosine peak currents were observed at the accumulation potential of -0.20 V (Figure 3B).

The peak current (I_p) for the surface-adsorbed didanosine is directly proportional to the scan rate (ν) (figure 3C). A plot of I_p vs. ν was linear (correlation coefficient, 0.998), with a slope of 54.4, over the 10-200 mV s^{-1} .

The dependence of the linear cyclic current peak with the pre-concentration time was verified. For longer accumulation time, more didanosine is adsorbed and higher peaks current appears. Such time-dependent profiles represent the corresponding adsorption isotherms because the peak current depends on the amount adsorbed. Initially the current increases linearly (0-180 s), and then drops at longer accumulation times. The resulting plot of peak current vs. accumulation time (0-180 s) show a slope of 36.03 nA s^{-1} and a correlation coefficient of 0.996. Figure 4 shows two didanosine voltammograms with accumulation time of 120 (A) and 240 (B) seconds. With 240 s of pre-concentration time the peak current for 1.0 ppm of didanosine was about 2.1 times larger than the corresponding peak obtained with a 120 s response.

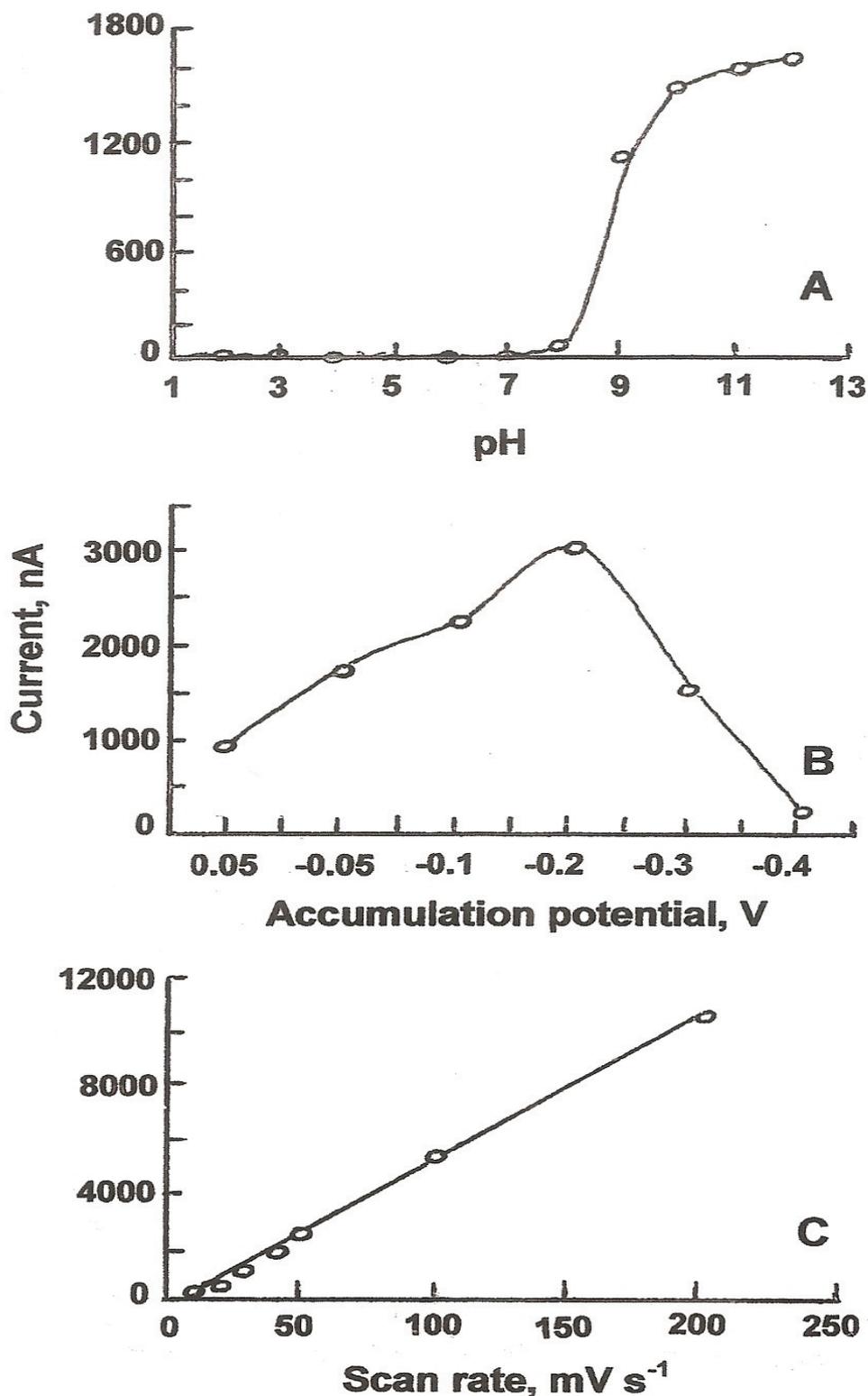


Figure 3. A - Effect of pH on the current peak height of the linear cyclic adsorptive stripping voltammograms of 1.0 ppm of didanosine in solution. Condition time, 60 s at -0.90 V; accumulation time 240 s at -0.30 V; final potential, -0.9 V; scan rate, 50 mV s⁻¹; equilibrium time, 30 s; thin-film mercury electrode (5 min at -0.9 V). B - Effect of accumulation potential on the current peak height of the linear cyclic adsorptive stripping voltammograms of 0.05 ppm didanosine in 2.0×10⁻³ mol L⁻¹ NaOH. Accumulation time, 120 s; scan rate, 100 mV s⁻¹. Other conditions same as 3A. C - Effect of scan rate on the current peak height of the linear cyclic voltammograms of 1.0 ppm didanosine in 2.0×10⁻³ mol L⁻¹ NaOH. Accumulation time, 240 s at -0.20 V. Other conditions same as 3A.

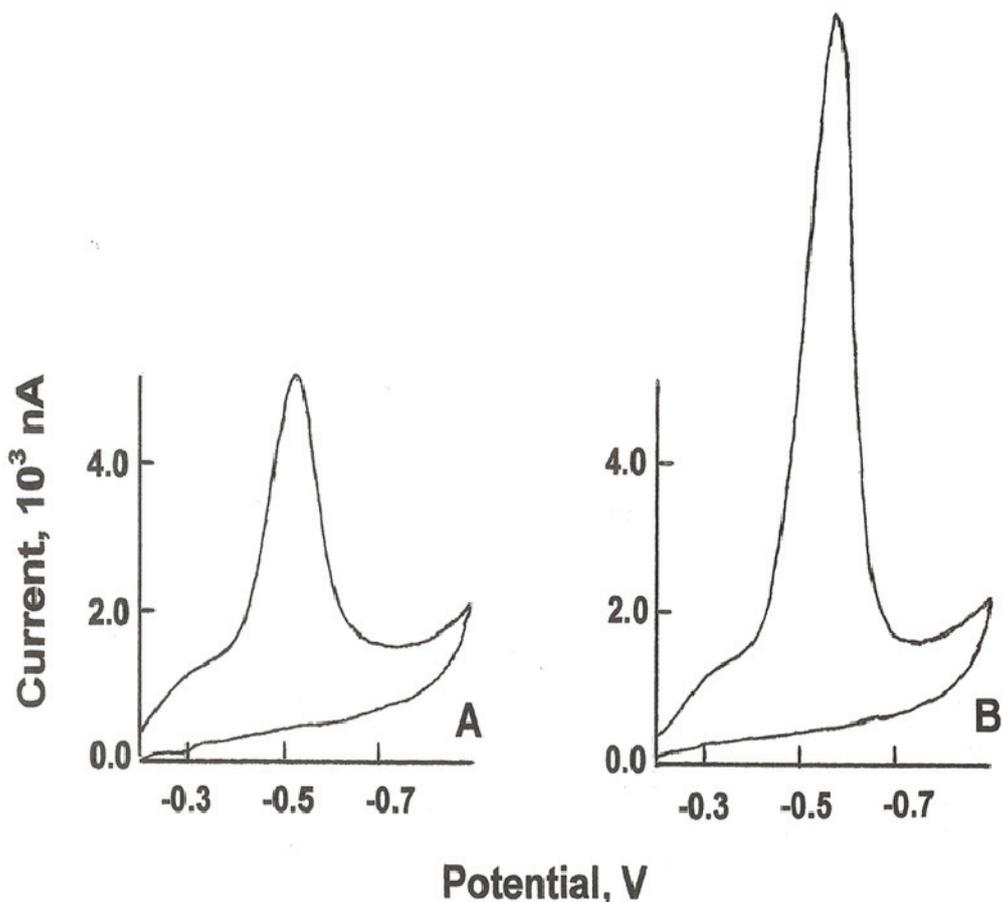


Figure 4. Increasing current effects of accumulation time at -0.20 V on the linear cyclic adsorptive stripping voltammograms of 1.0 ppm of didanosine in 2.0×10^{-3} mol L $^{-1}$ NaOH. The (A) and (B) curve is relative at accumulation time of 120 and 240 s. Conditioning time, 60 s at -0.90 V; scan rate, 100 mV s $^{-1}$; equilibrium time, 30 s; thin-film mercury electrode (5 min at -0.9 V).

The effective pre-concentration (accumulation time) associated with the adsorption process results in significant lowering of the detection limit compared to the corresponding solution measurements. A detection limit of 0.43 ppb (1.0×10^{-9} mol L $^{-1}$) was estimated from quantification of 0.01 ppm after 7 min accumulation ($S/N = 2$). Thus, 4 ng could be detected in the 10 mL of solution used.

The reproducibility was estimated by ten successive measurements in stirred 0.05 ppm didanosine solution (other conditions: supporting electrolyte, 0.002 mol L $^{-1}$ NaOH; conditioning time, 60 s at -0.9 V; accumulation time, 180 s at 0.2 V; final potential, -0.9 V; scan rate, 100 mV s $^{-1}$; equilibrium time, 30 s and thin-film mercury electrode). The mean peak current was 3255 nA with a range of $3111 - 3481$ nA and a relative standard deviation of 2.6% . Such precision compares similarly with those reported for other compounds measured by adsorptive stripping analysis [13-20]. The E_p and the $b_{1/2}$ remain the same at -0.56 V and 130 mV, respectively.

Figure 5 shows voltammograms for increasing didanosine concentration (A: 0.005 , B: 0.030 and C: 0.050 ppm) after 300 s accumulation. Well-defined stripping peaks (at -0.56 V) were observed from 0.005 to 0.050 didanosine concentration range. The resulting plot of peak current versus concentration is linear (slope 22550 nA ppm $^{-1}$; correlation coefficient, 0.992). Such linearity prevails as long as linear isotherm conditions (low surface coverage) exist. A separate experiment was performed to test linearity at high concentration range resulting also in well-defined stripping peaks between 0.2 and 0.4 ppm didanosine (accumulation time, 180 s at -0.3 V; scan rate, 50 mV s $^{-1}$; final

potential, -0.9 V; other conditions same as in Figure 5). The resulting plot of peak current versus concentration also showed linearity (slope 751 nA ppm⁻¹; correlation coefficient, 0.997).

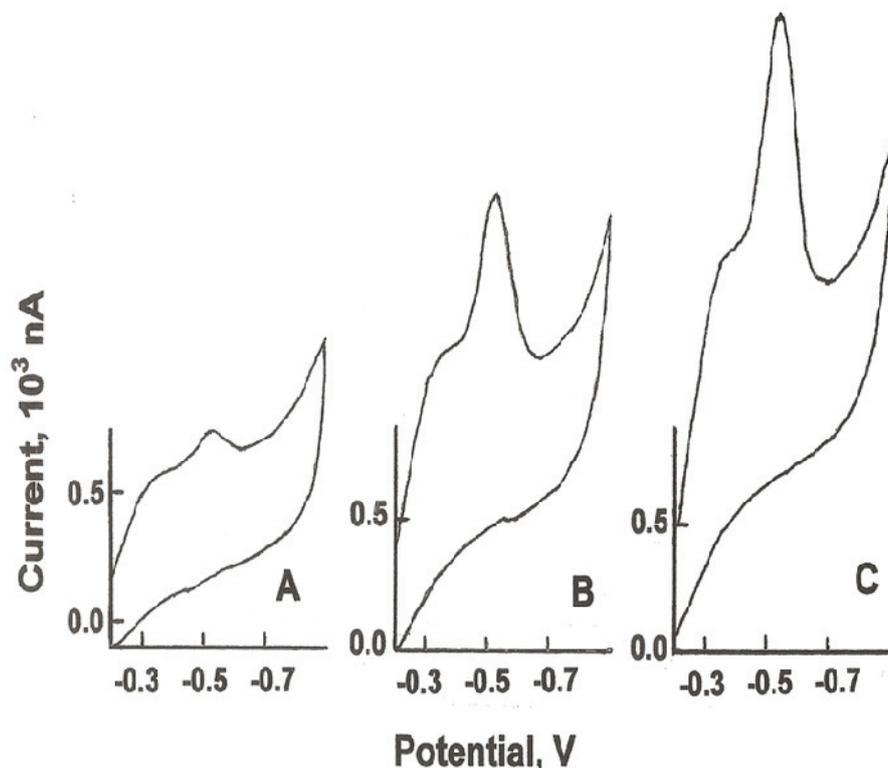


Figure 5. Linear cyclic scan adsorptive stripping voltammograms obtained after increasing the didanosine concentration in a solution of $2.0 \times 10^{-3} \text{ mol L}^{-1}$ NaOH (with 1% v/v of ethylic alcohol). The (A), (B) and (C) curve is relative at 0.005, 0.030 and 0.050 ppm of didanosine concentration. Accumulation time, 300 s at -0.20 V. Condition time, 60 s at -0.9 V. Scan rate, 100 mV s^{-1} . Final potential at -0.9 V. Equilibrium time, 30 s. Thin-film mercury electrode (5 min at -0.9 V).

Practical applications of differential pulse adsorptive stripping analysis may be affected by interferences due to the presence of ions and/or surface active compounds. With respect to the surface reaction, double layer charge or direct interactions among these substances may inhibit or aid in the accumulation of the analyte. Measurements of 0.05 ppm didanosine were not affected by addition of up to 0.01 cobalt(II); up to 0.06 ppm nickel(II) and up to 0.10 ppm cadmium(II), lead(II), zinc(II), copper(II) and iron(III). Using higher concentration a perceptible negative shift of didanosine peak was observed by presence of nickel (II) or cobalt (II) indicating a possible formation of Ni- or Co-didanosine complex. More dramatic interference was observed with cobalt(II). The addition of 0.03 ppm cobalt(II) resulted in a 5-fold increase of didanosine peak height. The metal ions chosen to be used in this study were based on their importance to human life. Cu, Fe, Co and Zn are essential while Pb and Cd are considered toxic to human life, since Ni is a genetic modifier. Preliminary studies were carried out for the determination of didanosine in presence of others antiretroviral drugs routinely used for the treatment of a human immunodeficiency virus (HIV): efavirenz, acyclovir, nevirapine, indinavir, nelfinavir, saquinavir, lamivudine and zidovudine. These antiretroviral drugs were chosen because they are produced in Brazil and have low costs. Measurements of 0.05 ppm didanosine were not affected by addition of up to 0.01 ppm of lamivudine or zidovudine; up to 0.03 ppm of nevirapine; up to 0.04 ppm of indinavir; up to 0.08 ppm of nelfinavir; up to 0.10 ppm of acyclovir and saquinavir. The more dramatic interference was observed with efavirenz. The addition of 0.03 ppm efavirenz resulted in

a twofold increase in didanosine peak height. A linear scan adsorptive stripping voltammogram for 0.1 ppm of free efavirenz in a 2×10^{-3} mol L⁻¹ NaOH solution showed a cathodic peak at -0.36V. A perceptible negative shift of didanosine peak was observed by presence of nelfinavir.

Figure 6 illustrates the method's suitability for the determination of didanosine through linear cyclic adsorptive stripping voltammetry in a synthetic sample contained contain several antiretroviral drugs (efavirenz, nevirapine, didanosine, lamivudine, zidovudine and acyclovir; all with 2.0 ppm of concentration). Four successive standard additions to sample resulted in well-shaped adsorptive stripping peaks. The didanosine peak in the original sample (curve A) can, thus, be quantified based on the resulting standard addition plot (also show in Figure 6). Because of the inherent sensitivity of the method, short (60 s) accumulation times could be used. Five consecutive analysis of sample yielded an average value of 2.5 ppm with standard deviation of the 0.4 ppm. This is in "relative" agreement with the didanosine value (2.0 ppm).

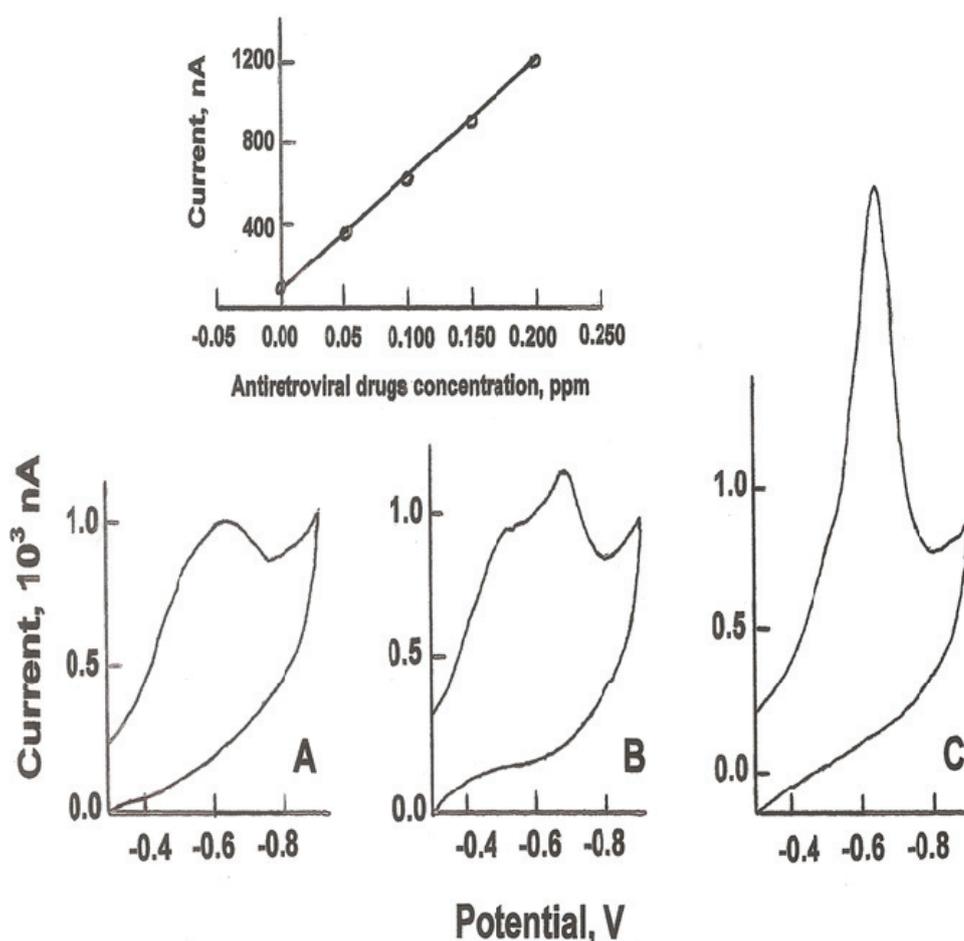


Figure 6. Illustration of didanosine determination in a synthetic sample containing several antiretroviral drugs (efavirenz, nevirapine, didanosine, lamivudine, zidovudine and acyclovir; all with 2 ppm of concentration) by linear cyclic adsorptive stripping voltammetry. Supporting electrolyte, 10 mL of 2.0×10^{-3} mol L⁻¹ NaOH. A - addition of 40 μ L of synthetic sample; B - 0.025 ppm and C - 0.20 ppm addition of standard didanosine. Condition time, 60 s at -0.9 V. Accumulation time, 60 s at -0.30 V. Final potential, -0.9 V. Scan rate, 100 mV s⁻¹. Equilibrium time, 30 s. Thin-film mercury electrode (5 min at -0.9 V).

Preliminary studies were also carried out for the determination of didanosine in the presence of hypoxanthine, ATP and DNA. The didanosine is a nucleoside analogue of adenosine [1-3]. The current measurements of 0.05 ppm didanosine (other conditions: supporting electrolyte,

2×10^{-3} mol L⁻¹ NaOH; condition time, 60 s at -0.9 V; accumulation time, 120 s at -0.2 V; final potential, -1.10 V; scan rate, 100 mV s⁻¹; the equilibrium time, 30 s and thin-film mercury electrode) were not affected by the addition of up to 0.10 ppm of DNA. In another experiment, after the addition of 0.10 ppm of DNA and copper(II) in 0.05 ppm of didanosine (with a potential peak at -0.56 V), a Cu(II) anodic and DNA cathodic peaks were observed at -0.20 and -0.75 V, respectively (Figure 7).

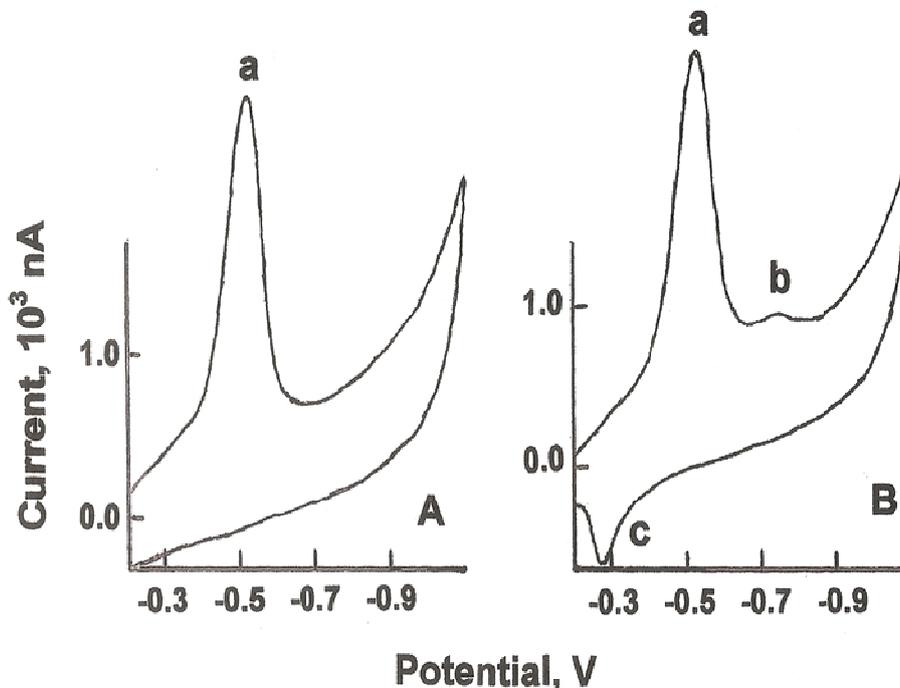


Figure 7. Linear cyclic pulse adsorptive stripping voltammogram of 0.05 ppm didanosine (A) in 0.10 ppm copper(II) and DNA presence (B) in a solution of 2.0×10^{-3} mol L⁻¹ NaOH. (a) Represent the didanosine, (b) the DNA and (c) the copper (II) peak. Accumulation time, 120 s at -0.20 V. Condition time, 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).

The current measurements of 0.05 ppm didanosine (other conditions: same as DNA experiment) were not affected by addition of up to 0.10 ppm of ATP. In another experiment, after the addition of 0.10 ppm of ATP and copper(II) in 0.05 ppm of didanosine (with a potential peak at -0.52 V), a Cu(II) anodic and ATP cathodic peak were observed in -0.15 and -0.75 V, respectively. The current measurements of 0.05 ppm didanosine (final potential, -1.20 V; other conditions the same as DNA experiment) were not affected by addition of up to 0.10 ppm of hypoxanthine. In another experiment, after the addition of 0.10 ppm of hypoxanthine and copper(II) in 0.05 ppm of didanosine (with a potential peak at -0.52 V), a Cu(II) anodic and hypoxanthine cathodic peaks were observed in -0.30 and -1.10 V, respectively. Without the didanosine presence, the hypoxanthine, ATP and DNA also showed similar behavior in presence of copper(II) [13-20].

Conclusions

An effective means for the determination of trace levels of didanosine has been described. The use of a diluted alkaline electrolyte provided a sensitive and selective adsorptive stripping voltammetric method for didanosine determination. Interference studies indicate the possibility of the determination of didanosine in presence of others antiretroviral drugs for the treatment of a human immunodeficiency virus (HIV), like acyclovir, nevirapine, indinavir, nelfinavir and

saquinavir. In particular, this approach offers similar efficiency to chromatographic methods. The didanosine peak increases in the presence of nickel(II) or cobalt(II) indicating a possible formation of Ni or Co-didanosine complex. The didanosine with the DNA peak are separated by 0.19 V and with the ATP by 0.23 V. Also, further studies using diluted alkaline solution as supporting electrolyte and film mercury electrode modified *in situ* by metallic ions can be used for detection of other drugs, and DNA-intercalating dyes, as well as amino-acids, peptides and proteins determinations.

Acknowledgements: *The authors gratefully acknowledge the CAPES-Brazil and MES-Cuba for their support of this work. In addition, we thank Dr. Katia Cristina Leandro of Fundação Oswaldo Cruz for generously supplying the sample of didanosine.*

References

- [1] E. De Clercq, *J. Clin. Virol.* **30** (2004) 115-133.
- [2] E. De Clercq, *Int. J. Biochem. Cell B.* **36** (2004) 1800-1822.
- [3] Sweetman, In: S.C. Sweetman, Editor, *Martindale the Complete Drug Reference* (35 th ed.), Pharmaceutical Press (2007).
- [4] R.D.E. Estrela, M.C. Salvadori, R.S.L. Raices, G. Suarez-Kurtz, *J.Mass. Spectrom.* **38** (2003) 378-385.
- [5] Y.Huang, E. Zurlinden, E. Lin, X.H. Li, J. Tokumoto, J. Golden, A. Murr, J. Engstrom, J. Conte, *J. Chromatogr.B* **799** (2004) 51-61.
- [6] A.M.C. de Oliveira, T.C.R. Lowen, L.M. Cabral, E.M. dos Santos, C.R. Rodrigues, H.C. Castro, T.C. dos Santos, *J.Pharmaceut. Biomed.* **38** (2005) 751-756.
- [7] C.P.W.G.M. Verweij-van Wissen, R.E. Aarnoutse, D.M. Burger, *J. Chromatogr.B* **816** (2005) 121-129.
- [8] T.N. Clark, C.A. White, M.G. Bartlett, *Biomed. Chromatogr.* **20** (2006) 605-611.
- [9] A. S. Kumari, K. Prakash, K. E. V. Nagoji, M. E. B. Rao, *Asian J. Chem.* **19** (2007) 2633-2636.
- [10] S. Notari, C. Mancone, T. Alonzi, M. Tripodi, P. Narciso, P. Ascenzi, *J. Chromatogr.B* **863** (2008) 249-257.
- [11] C.V. Kumar, D. Anantantahakumar, K. Madhuri, J.V.L.N.S. Rao, V.L.N. Seshagiri, *Asian J. Chem.* **23** (2011) 583-586.
- [12] K.I. Ozoemena, R.I. Stefan-van Staden, T. Nyokong, *Electroanal.* **21** (2009) 1651-1654.
- [13] P.A.M. Farias, A. de L.R. Wagener, A.A. Castro, *Anal. Let.* **34** (2001) 2125-2140.
- [14] P.A.M. Farias, A. de L.R. Wagener, A.A. Castro, *Anal. Let.* **34** (2001) 1295-1310.
- [15] P.A.M. Farias, A. de L.R. Wagener, A.A. Junqueira, A.A. Castro, *Anal. Let..* **40** (2007) 1779-1790.
- [16] P.A.M. Farias, A.A. Castro, Wagener, A.de L.R., A.A. Junqueira, *Electroanal.* **19** (2007) 1207-1212.
- [17] P.A.M. Farias, K.C. Leandro, J.C. Moreira, *Anal. Let..* **43** (2010) 1951-1957.
- [18] A.A.Castro, M.V.N. de Souza, N.A. Rey, P.A.M. Farias, *J. Braz.Chem. Soc.* **22** (2011) 1662-1668.
- [19] A.I.P. Cordoves, P.A.M. Farias, *Curr. Pharm. Anal.* **7** (2011) 71-78.
- [20] A.A. Castro, R.Q. Aucelio, N.A. Rey, E.M. Miguel, P.A.M. Farias, *Comb. Chem. High T.Scr.* **14** (2011) 22-27.