

## Short Communication

## Metallophthalocyanine Based Carbon Paste Electrodes for the Determination of 2',3'-Dideoxyinosine

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Received: February 17, 2009

Accepted: March 24, 2009

**Abstract**

Novel electrochemical sensors based on carbon paste impregnated with metallophthalocyanine (MPc, M = Co, Fe) complexes, have been constructed for the assay of anti-HIV drug 2',3'-dideoxyinosine (didanosine, DDI). Both modified electrodes showed electrocatalytic activity towards the oxidation of dideoxyinosine in phosphate buffer pH 7.4 with a working concentration range of  $10^{-6}$ – $10^{-4}$  mol/L and a detection limit of  $10^{-7}$  mol/L magnitude order. The sensor proved to be highly reliable for the assay of the purity of DDI - raw material as well as for the uniformity content test of Videx tablets.

**Keywords:** Cobalt(II) and Iron(II) phthalocyanines; Differential pulse voltammetry; 2',3'-Dideoxyinosine, Sensors, Drug design

DOI: 10.1002/elan.200904581

2',3'-Dideoxyinosine (Didanosine, DDI) (Fig. 1) is a dideoxy analogue of the purine nucleoside inosine, which shows high anti-retroviral activity against human immunodeficiency virus (HIV), the agent of acquired immunodeficiency syndrome (AIDS) [1, 2]. Dideoxyinosine was approved as a drug in 1991 by the Food and Drug Administration for the treatment of adults and children with advanced HIV infection, who are intolerant or resistant to azidothymidine (AZT) therapy or whose health has been deteriorated by AZT use [3]. Dideoxyinosine is useful both as a monotherapy and a combination therapy with other anti-HIV drugs in patients at different stages of HIV disease [1–3].

Direct detection of purine-containing nucleotides is possible [4], however, up to now, the determination of dideoxyinosine has been based on chromatographic and

capillary electrophoretic techniques. These include high performance liquid chromatography [5–8], capillary electrophoresis-electrospray ionization tandem mass spectrometry [9] and liquid chromatography-tandem mass spectrometry [10]. The use of the above techniques is expensive, laborious, time-consuming, requiring several harmful and undesirable reagents and chemical pre-treatments of samples. In contrast, electrochemical techniques offer advantages such as simple sample preparation, easily interpretable data for quantification at low levels, simplicity, high efficiency and low-cost of instrumentation [11, 12]. Differential pulse voltammetry (DPV) is an effective electroanalytical technique with well-known advantages, including good discrimination against background currents and low detection limits [11, 13].

Metallophthalocyanines (MPcs) have been applied in industrial (catalysts, photoconductors, etc) and biomedical (e.g. photodynamic therapy, PDT) fields [14]. Cobalt(II) phthalocyanine (CoPc) and iron(II) phthalocyanine (FePc) complexes have been found very attractive as useful electrocatalysts for the oxidation and reduction of a wide range of chemical processes [15–19]. Since the introduction of the use of CoPc based carbon paste electrode (CoPc-CPE) by Baldwin and co-workers [15], there have been several articles on the use of MPc based carbon paste electrodes (MPc-CPEs) for the detection and quantification of a wide range of chemical substances [16–17]. The analytical performance of such electrodes increased sub-

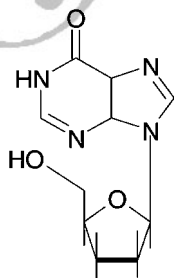


Fig. 1. Molecular structure of 2',3'-Dideoxyinosine (DDI).

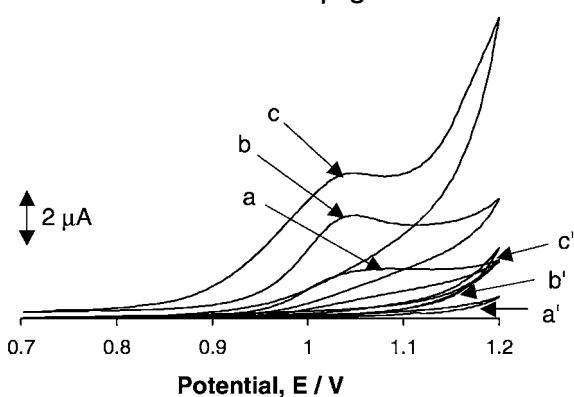


Fig. 2. Typical cyclic voltammograms at a) carbon paste electrode, b) carbon paste electrode modified with CoPc, and c) carbon paste electrode modified with FePc in the presence of  $8.0 \times 10^{-4}$  mol/L DDI. Cyclic voltammograms a'), b'), and c') were obtained for carbon paste electrode and carbon paste electrodes modified with CoPc and FePc, respectively, in the absence of DDI. Phosphate buffer pH 7.4; scan rate =  $25 \text{ mV s}^{-1}$ .

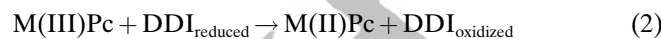
stantially through electrocatalytic activity or accumulation of analyte components. This leads to the lowering of detection limit of the species of interest and/or in increasing the selectivity for a certain analyte.

In this communication, we describe the construction and application of electrochemical sensors based on cobalt (CoPc) and iron (FePc) phthalocyanine complexes impregnated on carbon-paste matrices. The analytical application of the sensors is assessed by determining dideoxyinosine purity in raw material as well as the content uniformity of Videx tablets.

Figure 2 presents typical cyclic voltammograms (CV) of  $8.0 \times 10^{-4}$  mol/L 2',3'-dideoxyinosine at carbon paste electrode (Fig. 2a) and carbon paste electrodes modified with CoPc (Fig. 2b) and FePc (Fig. 2c) in pH 7.4 phosphate buffer solution at a scan rate of  $25 \text{ mV s}^{-1}$ .

As observed, the carbon paste electrodes modified with the CoPc and FePc complexes showed voltammograms with well-defined peak currents (at peak potential,  $E_p \approx 1.04 \text{ V}$  vs. Ag/AgCl) corresponding to the anodic oxidation of dideoxyinosine, whereas that for the bare carbon paste electrode tended to plateau at a slightly more positive potential value ( $E_p \approx 1.07 \text{ V}$  vs. Ag/AgCl). A lower peak current also noticed in the case of the bare carbon paste electrode indicates poor activity for dideoxyinosine at this electrode. From the figure, it is clear that FePc-CPE exhibited the best electrocatalytic behavior towards anodic oxidation of dideoxyinosine with lowest onset potential (0.85 V) compared to the CoPc-CPE (0.95 V) or bare CPE (0.98 V). Also, FePc-CPE gave higher catalytic current than either CoPc-CPE or the bare CPE. This is not surprising since FePc and CoPc have been known to exhibit different catalytic activity toward same analyte [15, 19]. No cathodic peak was observed on the reverse scan at any of the electrodes, which indicates the irreversible nature of the oxidation processes of nucleosides [20]. The anodic peak currents ( $I_{pa}$ ) of dideoxyinosine increases linearly with the

square root of scan rates ( $v$ ) within the range of  $5\text{--}300 \text{ mV s}^{-1}$  (not shown), which indicates a diffusion-controlled reaction process. In general, the CV results demonstrate the effective catalytic behavior of the CoPc and FePc complexes towards dideoxyinosine. M(II)Pc (M = Co or Fe) is known to be capable of oxidation to M(III)Pc [15, 17–19], which makes the oxidized MPc on the electrode capable of accepting electron from dideoxyinosine, regenerating the initial M(II)Pc, hence the catalytic activity (as summarized in Equations 1 and 2).



The practical usage of the proposed electrodes was determined using differential pulse voltammetry (DPV) measurements of dideoxyinosine. As exemplified in Figure 3, clearly defined DPV signals were obtained. The peak heights were measured at 1.02 and 0.994 V (vs. Ag/AgCl) for CoPc-CPE and FePc-CPE, respectively. The lower

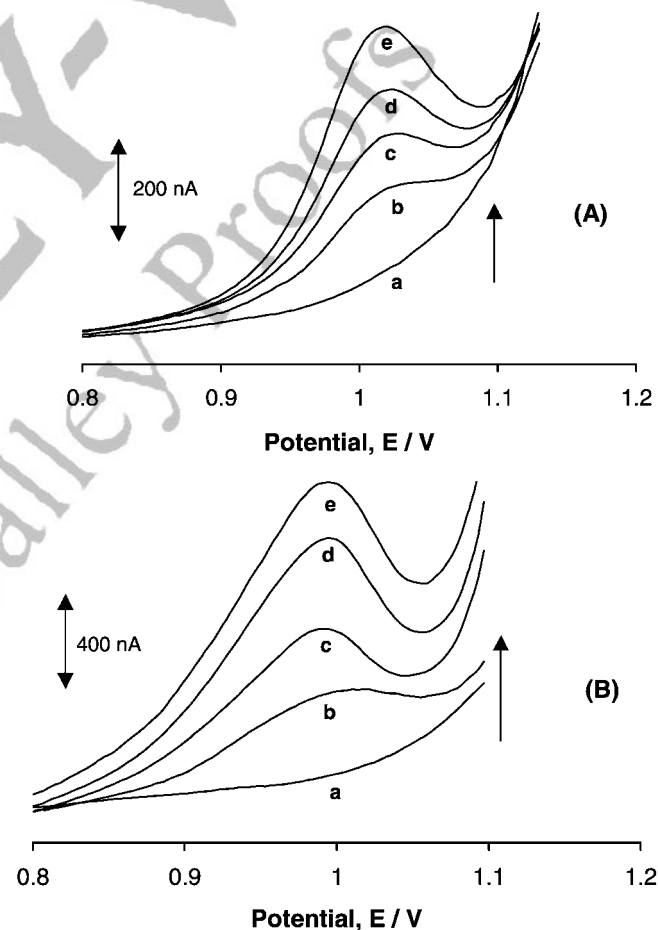


Fig. 3. Typical differential pulse voltammograms obtained at CoPc-CPE (A) and FePc-CPE (B) showing the increase of peak currents with increasing DDI concentrations a) 0, b) 50, c) 75, d) 95, and e)  $125 \mu\text{mol/L}$  DDI. Phosphate buffer pH 7.4; scan rate =  $25 \text{ mV s}^{-1}$ .

potential exhibited by the FePc-CPE shows that FePc catalyses dideoxyinosine easier than CoPc.

The responses of the electrodes to different dideoxyinosine concentrations were linear and showed the following calibration equations:

$$\text{CoPc-CPE: } I_p = 0.0048 + 0.0032 C; r = 0.9991$$

$$\text{FePc-CPE: } I_p = -0.0022 + 0.0063 C; r = 0.9988$$

where  $I_p$  is the peak current ( $\mu\text{A}$ ),  $C$  is the concentration of the dideoxyinosine ( $\mu\text{mol/L}$ ), and  $r$  is the regression coefficient. All the values are the average of ten measurements performed during one month. The calibration equations clearly show that FePc-based electrode is twice more sensitive than its CoPc counterpart. The detection limits ( $DL$ ) were calculated according to Otto [21]:

$$DL = (I_B + 3 \sigma_s - a) S^{-1}$$

where  $I_B$  is the background current recorded,  $\sigma_s$  is the standard deviation for the measurement of the background current,  $a$  is the intercept of the calibration equation, and  $S$  is the slope of the calibration equation. The detection limits were calculated to be  $8.80 \times 10^{-7} \text{ mol/L}$  for the CoPc-CPE with a linear concentration range between  $2.0 \times 10^{-6}$  and  $7.0 \times 10^{-4} \text{ mol/L}$ , whereas for the FePc-CPE the limit of detection is  $3.50 \times 10^{-7} \text{ mol/L}$  with a linear concentration range between  $1.0 \times 10^{-6}$  and  $8.0 \times 10^{-4} \text{ mol/L}$ . The  $RSD$  (%) values for the current intensity were lower than 2% during the one month test period. The proposed electrodes were highly stable and reproducible over a month test period. The reliability and stability of the new sensors are due to the excellent electrocatalytic and thermal stability properties of the MPc complexes [13].

The sensors proved to be useful for the content uniformity test of Videx tablets (Table 1). The results indicate that the average recoveries ( $99.76 \pm 0.10$  for CoPc-CPE and  $99.79 \pm 0.12$  for FePc-CPE) are quite comparable when using either CoPc or FePc complex as electrode modifier. The results are in good agreement and within the range given in the United States Pharmacopoeia XXIV [22], 90 to 110% per tablet.

In conclusion, metallophthalocyanine based carbon paste electrodes described in this paper provide good reproducibility for the determination of didanosine raw material and from its pharmaceutical formulation. The construction of the electrodes is simple, fast, highly reproducible and robust. The robustness and reliability of the analytical information can be assured by the low  $RSD$  values obtained in the recovery tests. The main advantage of the proposed method over the other known methods described for dideoxyinosine is the possibility of its determination directly, without prior separation, with high precision, rapidity, stability, and low consumption of sample and buffer. In addition, the proposed sensors can be a good substitute for the use of the expensive and less stable over a long period of time, amperometric immunosensors.

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Table 1. Results obtained from the assay of didanosine in Videx tablets (100 mg dideoxyinosine tablet) using amperometric sensor based on cobalt(II) (CoPc) and iron(II) (FePc) phthalocyanine complexes. All measurements were made at  $25^\circ\text{C}$ . All values are averages from ten measurements performed during one month.

Videx tablets sample	Recovery of dideoxyinosine (mg)	
	CoPc	FePc
1	$99.81 \pm 0.08$	$99.83 \pm 0.10$
2	$99.78 \pm 0.10$	$99.80 \pm 0.11$
3	$99.69 \pm 0.11$	$99.76 \pm 0.12$
4	$99.76 \pm 0.10$	$99.78 \pm 0.12$
5	$99.80 \pm 0.08$	$99.79 \pm 0.11$
6	$99.78 \pm 0.08$	$99.80 \pm 0.10$
7	$99.71 \pm 0.10$	$99.73 \pm 0.13$
8	$99.80 \pm 0.09$	$99.81 \pm 0.10$
9	$99.74 \pm 0.11$	$99.75 \pm 0.13$
10	$99.76 \pm 0.11$	$99.83 \pm 0.11$

### Experimental

2',3'-Dideoxyinosine was obtained from Sigma-Aldrich (St. Louis, MO, USA). Graphite powder (1–2  $\mu\text{m}$ , synthetic), CoPc and FePc were supplied by Aldrich. Paraffin oil was supplied by Fluka (Buchs, Switzerland). Videx tablets were obtained from Bristol-Myers Squibb. Deionized water obtained from a Modulab system (Continental Water Systems, San Antonio, TX, USA) was used for the preparation of all solutions. All solutions were buffered with phosphate buffer solution pH 7.4.

Cyclic and differential pulse voltammograms were recorded with a 663 VA stand (Metrohm, Herisau, Switzerland) connected to a PGSTAT 100 and a Eco Chemie (Utrecht, The Netherlands) software version 4.9 were used for all amperometric measurements. A platinum electrode and a Ag/AgCl (0.1 mol/L KCl) electrode served as counter and reference electrodes in the cell, respectively. The operating conditions for the differential pulse voltammetry were: applied potential pulse amplitude,  $25 \text{ mV s}^{-1}$ ; potential range, from 0.80 to 1.2 V (vs. Ag/AgCl); equilibration time, 5 s; step potential, 1.95 mV; pulse duration, 50 ms and pulse period, 0.2 s.

Graphite powder and MPc (4% based on the weight of graphite powder) were first ground together to ensure uniform dispersion of the phthalocyanine complex. This was followed by mixing paraffin oil and graphite-MPc mixture in a ratio of 1:4 (w/w). A certain quantity of graphite paste free of MPc was prepared and was placed into a plastic pipette peak leaving 3 to 4 mm empty in the top to be filled with carbon paste that contains the MPc. The diameter of the electrochemical sensor was 3 mm. Electric contact was obtained by inserting a Ag/AgCl wire into the carbon paste. The internal solution was 0.1 mol/L KCl. The surface of the electrode was wetted with deionized water and polished with alumina paper (polishing strips 30144-001, Orion) before using. When not in use, the electrochemical sensor was stored in a dry state at room temperature.

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The technique used for direct voltammetric assay was DPV with an applied pulse potential of 25 mV vs. Ag/AgCl. The metallophthalocyanine-carbon paste electrode (MPc-CPE), together with the reference and counter electrodes were dipped into the cell containing the buffered analyte. All solutions were deoxygenated with pure nitrogen for 5 minutes before the measurements. The peak heights were measured at 1.02 and 0.994 V (vs. Ag/AgCl) for CoPc-CPE and FePc-CPE, respectively and were plotted against the concentrations of the dideoxyinosine. The unknown concentrations of dideoxyinosine were obtained from the calibration graphs.

Each of ten Videx tablets (100 mg 2',3'-dideoxyinosine/tablet) was placed in a 100 mL volumetric flask and dissolved in phosphate buffer. The apparatus cell was filled with the prepared solution and the current developed was measured. The unknown concentration was determined from the calibration graphs as described in the direct differential pulse voltammetric method.

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