

## Short Communication

# Differential Pulse Polarographic Investigation of the Nickel(II)–Orotic Acid Complex

L. Calvo Blázquez, E. Pinilla Gil, R. M. García-Moncó Carra, and A. Sánchez Misiego\*

Departamento de Química Analítica y Electroquímica, Universidad de Extremadura, E-06071 Badajoz, Spain

Received: February 7, 1993

Final version: May 24, 1993

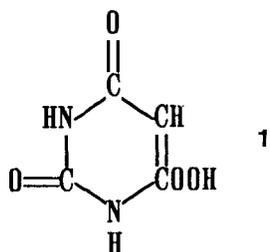
## Abstract

Differential pulse polarographic studies illustrate that the Ni<sup>II</sup> ion forms a complex with orotic acid (aqueous medium, pH = 9.5, ionic strength  $\mu = 0.2$ ). The stoichiometric composition of this complex has been determined, and a method for its determination in an aqueous medium is proposed.

**Keywords:** Orotic acid, Nickel complex, Pulse polarography

## 1. Introduction

Pyrimidine derivatives constitute a very important class of compounds as components of nucleic acids, and have been shown to exert a pronounced physiological effect. Since most living systems need metal ions, which are essential for proper functioning, an important question arises about the effect of such metal ions on nucleic acids. Orotic acid (1,2,3,6-tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic acid), **1**, a uracil derivative also known as vitamin B<sub>13</sub>, is one of the most significant pyrimidine derivatives with a biological function.



Heath [1] was probably the first to examine the polarographic reducibility of pyrimidine derivatives and Icha [2] described the general polarographic behavior of orotic acid. In previous papers [3, 4] the polarographic behavior of orotic acid under differential pulse polarography is described, studying both the reduction and oxidation processes. There are numerous papers that describe the occurrence of polarographic prewaves of the Ni<sup>II</sup> ion in the presence of sulfur- or nitrogen-containing organic compounds [5–9].

Kapetanovic and Milovanovic [10] studied the Ni<sup>II</sup>-5-hydroxytryptophan complex, obtaining a 1:1 metal-to-ligand ratio. Suznjevic and Kapetanovic [11] studied the nature of polarographic precursors of the Ni<sup>II</sup> ion in the presence of ampicillin, as well as the behavior of ampicillin in the electrical double layer. The polarographic behavior of an orotic acid–Cu<sup>II</sup> complex has been investigated previously by our group [12]. The best medium was found to be a pH 5.5 acetic acid–acetate buffer. This communication describes the polarographic study of the nature of the wave of the Ni<sup>II</sup> ion in the presence of orotic acid.

## 2. Experimental

### 2.1. Instrumentation

A PAR 384B polarograph was used, connected to a 303A polarographic stand, with the following electrodes: DME (dropping mercury electrode) working electrode, Ag/AgCl/saturated KCl reference electrode, and a glassy carbon auxiliary electrode. The dropping time was kept mechanically at 1 s.

### 2.2. Reagents

The Ni(NO<sub>3</sub>)<sub>2</sub>, orotic acid (Sigma Chemical Co., St Louis), NH<sub>4</sub>OH, and NH<sub>4</sub>Cl used were all analytical reagents. A stock solution of  $1.7 \times 10^{-2}$  mol/L Ni(NO<sub>3</sub>)<sub>2</sub> was standardized by a complexometric method. All of the experiments were performed in buffered media, where the pH was adjusted by adding NH<sub>4</sub>OH/NH<sub>4</sub>Cl buffer solution. Constant ionic strength was obtained by means of the buffer, and any oxygen was removed by a nitrogen flow. During recording, a nitrogen atmosphere was maintained above the solution.

## 3. Results and Discussion

Several differential pulse polarograms of a  $5 \times 10^{-5}$  mol/L Ni<sup>II</sup> solution before and after the addition of orotic acid, where the orotic acid concentration is increasing (all in pH = 9.5, 0.2 mol/L NH<sub>4</sub>OH/NH<sub>4</sub>Cl), are presented in Figure 1. Besides the reduction peak of Ni<sup>II</sup> (peak potential,  $E_p = -0.9$  V) (polarograms "A"), a new peak at  $E_p = -1.1$  V appears in the presence of orotic acid (polarograms "B"), probably due to the reduction of an Ni<sup>II</sup>-orotic acid complex formed in the solution. The Ni<sup>II</sup> peak disappears at an orotic acid concentration above  $2 \times 10^{-4}$  mol/L, but the complex peak potential is not affected. The complex solutions were found to be stable for at least five hours after preparation, since the polarographic response was the same during that period.

The effect of pH in the interval between 8.0 and 11.0 pH units was first investigated using NH<sub>4</sub>OH/NH<sub>4</sub>Cl buffer solution as the supporting electrolyte. This pH range was selected because the complex is only stable in an alkaline medium, and the best

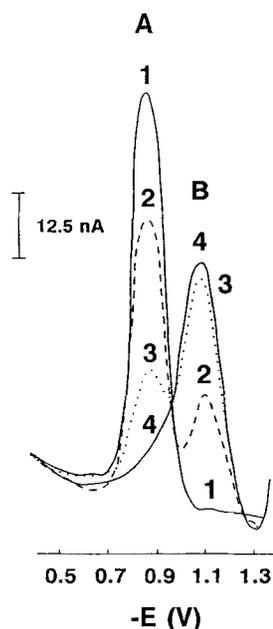


Fig. 1. Differential pulse polarography response of 1)  $5 \times 10^{-5}$  mol/L  $\text{Ni}^{\text{II}}$ , 2) (1) plus  $2 \times 10^{-5}$  mol/L orotic acid, 3) (1) plus  $6 \times 10^{-5}$  mol/L orotic acid, and 4) (1) plus  $2 \times 10^{-4}$  mol/L orotic acid. Polarograms A:  $\text{Ni}^{\text{II}}$  response, polarograms B: complex response. All curves obtained in pH 9.5, 0.2 mol/L,  $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$  buffer solution. Sweep rate: 10 mV/s,  $T$ : 25°C, pulse amplitude: -20 mV, drop time: 1 s.

wave is achieved in ammonia buffer. The reasons for this behavior could imply complexing properties of the buffer, but they were not investigated further. The reduction wave of the  $\text{Ni}^{\text{II}}$ -orotic acid complex suffers a displacement to more negative potentials when the pH increases, but the peak height is not affected. A pH value of 9.5 was selected for future experiments. In order to establish the nature of the new wave, the effect of drop time ( $t_d$ ), pulse amplitude, and  $\text{Ni}^{\text{II}}$  concentration on its peak height were sequentially investigated.

When the concentration of orotic acid is somewhat higher (about  $2 \times 10^{-4}$  mol/L) than the  $\text{Ni}^{\text{II}}$  concentration (about  $10^{-5}$  mol/L), the complex peak height is linearly dependent on the drop time. This data indicated that the complex's reduction process is diffusion controlled. A study of the influence of pulse

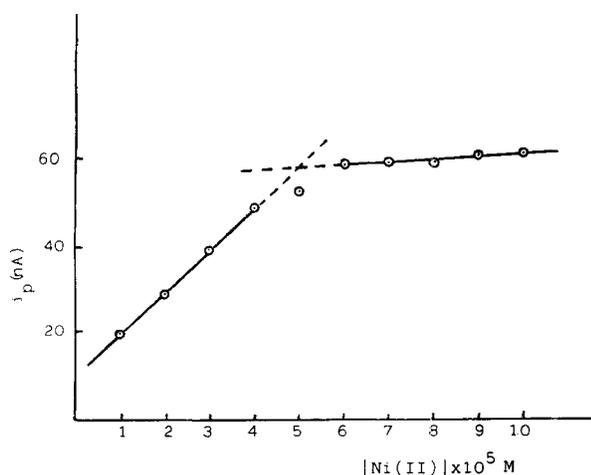


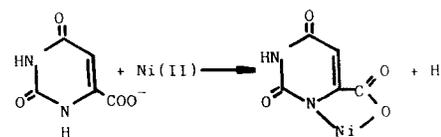
Fig. 2. Amperometric titration curve. Orotic acid concentration:  $5 \times 10^{-5}$  mol/L;  $\text{Ni}^{\text{II}}$  concentration varies from  $10^{-5}$  to  $10^{-4}$  mol/L. All other conditions as in Figure 1.

amplitude ( $\Delta E$ ) for a  $10^{-5}$  mol/L  $\text{Ni}^{\text{II}}$  solution and  $2 \times 10^{-4}$  mol/L orotic acid in pH = 9.5  $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$  buffer was then carried out. A linear relationship between the peak current,  $i_p$  and  $\Delta E$  was found when  $\Delta E$  was changed between -10 mV and -100 mV. This confirmed that the complex wave is diffusion controlled.

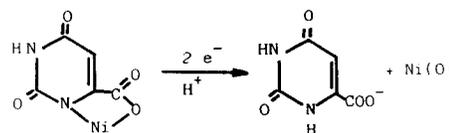
The metal-to-ligand ratio in the complex was determined by amperometric titration of  $\text{Ni}^{\text{II}}$  with a  $5 \times 10^{-5}$  mol/L orotic acid solution in pH 9.5  $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$  buffer. A titration curves showing the influence of the  $\text{Ni}^{\text{II}}$  concentration on the complex wave height are shown in Figure 2. The cross-section point, which was obtained by extrapolating the initial and final straight lines, indicated a 1:1 metal-to-ligand ratio.

A linear correlation was found between the peak current of the complex wave and the  $\text{Ni}^{\text{II}}$  concentration ( $5 \times 10^{-6}$ – $10^{-4}$  M) in pH 9.5  $\text{NH}_4\text{OH}/\text{NH}_4\text{Cl}$  buffer, and with  $5 \times 10^{-4}$  mol/L orotic acid. The linear correlation coefficient was 0.9998. Statistical calculations on several series of eleven identical samples containing  $5 \times 10^{-4}$  mol/L orotic acid and two different

1. - Formation of the Ni(II)-orotic acid complex.



2. - Electrochemical reduction.



Scheme 1.

$\text{Ni}^{\text{II}}$  concentrations ( $10^{-5}$  and  $5 \times 10^{-5}$  mol/L) gave a relative standard deviation of 2.9% and 1.2%, respectively. In concordance with the results obtained, a new amperometric method to determine  $\text{Ni}^{\text{II}}$  in the range from  $5 \times 10^{-6}$  to  $10^{-4}$  mol/L is proposed. The proposed mechanism for the process is shown in Scheme 1.

## 4. Conclusions

Orotic acid gives rise to a cathodic wave on the dropping mercury electrode in the presence of  $\text{Ni}^{\text{II}}$  due to reduction of the  $\text{Ni}^{\text{II}}$ -orotic acid complex with stoichiometry 1:1. The wave is diffusion controlled. Measurement of the complex peak height permits the polarographic determination of  $\text{Ni}^{\text{II}}$  in the range from  $5 \times 10^{-6}$  to  $10^{-4}$ , with a relative standard deviation (R.S.D.) of 2.9% at the  $10^{-5}$  M level.

## 5. References

- [1] J.C. Heath, *Nature* **1946**, 158, 23.
- [2] F. Icha, *Pharmazie* **1959**, 14, 684.
- [3] L. Calvo, J. Rodríguez, F. Vinagre, A. Sánchez, *Anal. Lett.* **1989**, 22, 177.
- [4] L. Calvo Blázquez, J. Rodríguez Flores, F. Vinagre Jara, A. Sánchez Misiego, *Proc. Indian Acad. Sci. (Chem. Sci)* **1989**, 101, 415.
- [5] Y.I. Turyan, *Zh. Fiz. Khim.* **1957**, 31, 2423.
- [6] H.B. Mark, *Anal. Chem.* **1964**, 36, 940.
- [7] H.B. Mark, *J. Electroanal. Chem.* **1964**, 7, 276.
- [8] Y.I. Turyan, Ruvinski, *Elektrokhimiya* **1968**, 4, 446.
- [9] Y.I. Turyan, O.N. Malyavinskaya, *Elektrokhimiya* **1969**, 5, 103.
- [10] V. Kapetanovic, L. Milovanovic, *Electroanalysis* **1989**, 1, 461.
- [11] D. Suznjec, V. Kapetanovic, *Electroanalysis* **1989**, 1, 457.
- [12] L. Calvo Blázquez, *Grad. Thesis*, Badajoz, Spain **1983**.