Journal of Pharmaceutical & Biomedical Analysis
Vol. 7, No. 10, pp. 1213–1216, 1989
Printed in Great Britain

0731–7085/89 $3.00 + 0.00

Short Communication

Voltammetric determination of 25-hydroxyvitamin D_3 with a rotating glassy carbon electrode

M. DELGADO ZAMARREÑO,* A. SÁNCHEZ PÉREZ, J. HERNÁNDEZ MÉNDEZ and F. A. MARTÍN DOMINGUEZ

Department of Analytical Chemistry, Nutrition and Bromatology, University of Salamanca, 37008 Salamanca, Spain

Keywords: *Voltammetry; 25-hydroxyvitamin D_3.*

Introduction

It is well known that 25-hydroxyvitamin D_3 (25-OH D_3) is the major circulating metabolite of vitamin D_3. The measurement of the serum levels of 25-OH D_3 in patients with disorders in vitamin D levels and bone mineral metabolism is of great clinical significance and offers an important analytical challenge. Gas chromatography (GC), high-performance liquid chromatography (HPLC), competitive protein binding assay, and radioimmunoassay techniques have been developed [1–15].

Recently, a voltammetric method for the analysis of vitamin D_3 was reported [16]. The present paper described the voltammetric behaviour of 25-OH D_3 as a means for determining the substance at low concentrations. The new method may be applied to the electroanalytical determination of 25-OH D_3 in samples of pharmaceutical interest. Knowledge of the voltammetric behaviour of vitamin D_3 and its metabolites should provide information for the analysis of these compounds by electrochemical detection after LC separation.

Experimental

Apparatus

A three electrode cell with a rotating glassy carbon electrode, previously described [16], a saturated calomel electrode (SCE) and a platinum counter electrode were used with the METROHM polarecord.

*To whom correspondence should be addressed.

Reagents

25-Hydroxyvitamin D_3 was kindly donated by Roussel Uclaf (Paris). Vitamin D_3 was supplied by Merck. Analytical grade lithium perchlorate and methanol were used.

Procedure

Methanolic solutions of 25-OH D_3 were prepared using 0.075 M lithium perchlorate as supporting electrolyte. The solutions under study were placed in the electroanalytical cell and the corresponding voltamperograms were recorded with a potential sweep from +0.8 to +1.8 V (vs SCE). Voltammetric curves were recorded in the differential pulse (DP) mode with a ΔE value of +50 mV. The electrode rotation rate was 1580 rpm. Treatment of the glassy carbon electrode was carried out as described previously [16].

Results and Discussion

Voltammetric behaviour of 25-hydroxyvitamin D_3

In the methanolic solution 25-OH D_3 exhibits a well-defined wave in the direct current mode (DC) or peak in the differential pulse mode (DP) at potentials close to +1.1 V (vs SCE), as previously described for vitamin D_3. A poorly-defined peak at potentials near to +1.3 V was observed in the voltammetric oxidation of 25-OH D_3 only when the differential pulse technique was used (Fig. 1).

The reproducibility of the voltammetric signal of a solution of 1.3×10^{-5} M of 25-OH D_3 was investigated because it was observed that continuous connection of the electrode assembly or successive potential sweeps gave rise to a gradual decrease in the voltammetric signal. The results show a smaller adsorption of 25-OH D_3 onto the electrode surface in comparison with vitamin D_3, as seen in the study of the reproducibility carried out with the potential sweep rate. A scan rate of 20 mV s^{-1} is recommended with the additional precaution of maintaining the working electrode connection only when each voltammetric curve is recorded.

No changes in the limiting current or peak intensities were observed when the concentration of lithium perchlorate, as supporting electrolyte, was varied over the 0.020–0.080 M range. A lithium perchlorate concentration of 0.075 M was chosen.

Temperature coefficients using the DC and DP techniques were also evaluated. The

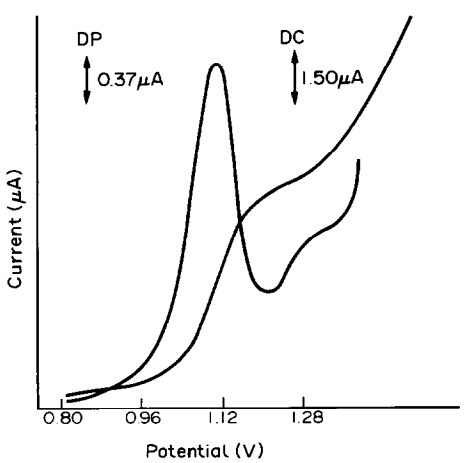

Figure 1
Electrochemical behaviour of 25-OH D_3.

values found, 2.03% (DC) and 0.77% (DP) per degree (for 5–39°C range), respectively, point to the predominance of the diffusional characteristics of the voltammetric process.

Determination of 25-hydroxyvitamin D_3

The relationship of the limiting current (DC) and peak intensity (DP) and the 25-OH D_3 concentration (1.12×10^{-6}–4.14×10^{-5} M range) was found to be linear

$$i_l\,(\mu A) = 0.0869 + 1.53 \times 10^5 C(M); \qquad r = 0.9992$$

$$i_p(\mu A) = 0.0733 + 8.35 \times 10^4 C(M); \qquad r = 0.9988.$$

These results show that the voltammetric curve or peak obtained at +1.1 V (vs SCE) in the voltammetric oxidation of 25-OH D_3 using a rotating glassy carbon electrode can be used for the quantitative determination of 25-OH D_3 over the 0.5–16 μg ml^{-1} range.

After applying the proposed method to 10 standard solutions, each containing 5.2 μg ml^{-1} of 25-OH D_3, the relative standard deviations (RSD) found were 2.3 and 4.3% using the direct current and differential pulse techniques, respectively.

Interference by vitamin D_3

Both vitamin D_3 and its metabolite 25-OH D_3 exhibit voltammetric peaks at +1.1 V. However, only 25-OH D_3 gives a DP peak at +1.3 V, which is poorly defined. Interference of vitamin D_3 in the determination of 25-OH D_3 was observed when increasing concentrations of vitamin D_3 (1.56×10^{-6} to 3.12×10^{-5} M) were added to a 1.48×10^{-5} M solution of 25-OH D_3. The first peak (E_p = +1.1 V) increases linearly and the measurement of the second peak (E_p = +1.3 V) becomes more difficult as the vitamin D_3 concentration increases (Fig. 2).

Analysis of 25-hydroxyvitamin D_3 in pharmaceutical products

25-Hydroxyvitamin D_3 is generally formulated in oily solutions for injection or oral administration. The voltammetric method was applied to the determination of the 25-OH D_3 content of two pharmaceutical products. The best results were obtained using DP voltammetry and the standard addition method. Differences of between −8 and −10% were found between the analytical results and the stated concentration of 25-OH D_3 (0.266 and 1 mg ml^{-1}, respectively).

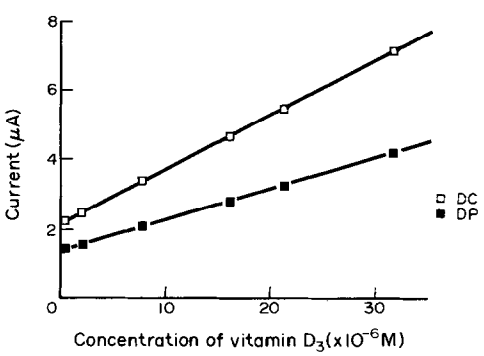

Figure 2
Interference of vitamin D_3 in the assay of 25-OH D_3 (1.48×10^{-5} M).

References

[1] B. Zagalak and F. Neuheiser, *Anal. Chem. Symp. Ser.* **14**, 347 (1983).
[2] H. L. J. Makin and D. J. H. Trafford, *Proc. Workshop Vitamin D* 823 (1985).
[3] K. T. Koshy and A. L. van der Bilk, *Analyt. Biochem.* **74**, 282–291 (1976).
[4] T. J. Gilbertson and R. P. Strayd, *Clin. Chem.* **23**, 1700–1704 (1977).
[5] J. A. Eisman, R. M. Shepard and H. F. de Luca, *Analyt. Biochem.* **80**, 298–305 (1977).
[6] G. Jones, *Clin. Chem.* **24**, 287–297 (1978).
[7] E. Mayer and G. H. Schimidt, *Clin. Chem.* **30**, 1199–1204 (1984).
[8] J. Briton and H. L. Wray, *Clin. Chem.* **31**, 783–784 (1985).
[9] L. Hummer and D. Hartwell, *Proc. Workshop Vitamin D* 817 (1985).
[10] R. L. G. Norris, M. J. Thomas and P. W. Craswell, *J. Chromatogr. Biomed. Appl.* **54**, 53–61 (1986).
[11] J. G. Haddad and K. J. Chyn, *J. Clin. Endocr.* **33**, 992 (1971).
[12] S. Edelstein, M. Charman, P. E. M. Lawson and E. Kodicek, *Clin. Sci. Molec. Med.* **46**, 231 (1974).
[13] J. F. Morris and M. Peakock, *Clin. Chim. Acta* **72**, 383–391 (1976).
[14] D. A. Seamark, D. J. H. Trafford and H. Makin, *Clin. Chim. Acta* **106**, 51–62 (1980).
[15] B. W. Hollis and J. L. Napoli, *Clin. Chem.* **31**, 1815–1819 (1985).
[16] J. Hernandez Mendez, A. Sanchez Perez, M. Delgado Zamarreño and M. L. Hernandez, *J. Pharm. Biomed. Anal.* **6**, 737–741 (1988).

[*Received for review 20 October 1988*]