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Voltammetric determination of vitamin D_3 with a rotating glassy carbon electrode*

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Abstract: A voltamperometric study (DC and DP) on the electroanalytical behaviour of vitamin D_3 in a methanolic solution using $LiClO_4$ as the supporting electrolyte and working with a glassy carbon electrode was carried out. Vitamin D_3 exhibits an oxidation wave (DC) or peak (DP) at potentials close to +1.1 V (versus SCE). The optimum experimental conditions for the best reproducibility of the voltamperometric signal were determined and the different parameters affecting the electrochemical process were studied. The electrochemical process was seen to be irreversible and, under certain conditions, adsorption of vitamin D_3 onto the electrode surface was observed. A voltamperometric procedure for the determination of vitamin D_3 in a concentration range of 2×10^{-6}–2×10^{-4} M is proposed. The detection limit is of the order of 2×10^{-6} M and the relative standard deviations are 1.1% (DC) and 2.6% (DP), respectively.

Keywords: *Voltammetry; vitamin D_3.*

Introduction

Application of modern polarographic and voltamperometric techniques to the analysis of vitamins has resolved many of the analytical problems that arise in the determination of these compounds in complex samples such as biological fluids, pharmaceutical products and foods (1). Although vitamins C and E have received the greatest attention, certain other vitamins such as folic acid, riboflavin, thiamine, pyridoxine and vitamin A (and derivatives) have also been determined by electroanalytical techniques [2, 3].

In spite of the biological importance of vitamin D and its derivatives there are few electroanalytical studies on such compounds. Outstanding is the work of Atuma *et al.* [4] who determined mixtures of vitamins A and D using a glassy carbon electrode. The D group vitamins are seco-9,10 steroids, vitamin D_3 (or cholecalciferol) being the natural form; 25-hydroxy-vitamin D_3 (25-OHD_3) is the active metabolite that results from the hydroxylation of vitamin D_3 in the liver and is the necessary circulating form for other transformations occurring in the kidney. Determination of the levels of 25-OHD_3 in serum is of great clinical interest and presents an important analytical challenge (5).

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Experimental

Apparatus

A METROHM E-506 polarograph, a METROHM EA-289/1 glassy carbon rotary working electrode and a METROHM EA-285 platinum auxiliary electrode were used. A saturated calomel electrode (SCE) was constructed in this Department. A P-SELECTA circulation thermostat was used.

Reagents

Pure vitamin D_3 was supplied by Merck. Analytical grade methanol and $LiClO_4$ were used.

Procedure

Methanolic solutions of vitamin D_3 at the desired concentrations were prepared using 0.075 M $LiClO_4$ as the supporting electrolyte. The solutions were placed in the electroanalytical cell and the corresponding voltamperograms were recorded performing a potential sweep in the +0.8–1.8 V range (versus SCE). The electrode rotation rate employed, except when the influence of this variable was studied, was 1580 r.p.m. When the differential pulse technique was used a ΔE value of +50 mV was applied.

The glassy carbon electrode was treated after tests on several of those described by Laser and Ariel [6]; after polishing, the electrode was placed in a solution containing methanol and $LiClO_4$ (background solution) and a potential of +1.4 V was applied for 5 min.

Results and Discussion

Studies on voltammetric techniques for determination of vitamin D_3

In the medium used (methanol and $LiClO_4$) vitamin D_3 exhibits a well-defined voltammetric wave (direct current) (DC) or peak (differential pulse) (DP) at potentials near +1.1 V (versus SCE) (Fig. 1).

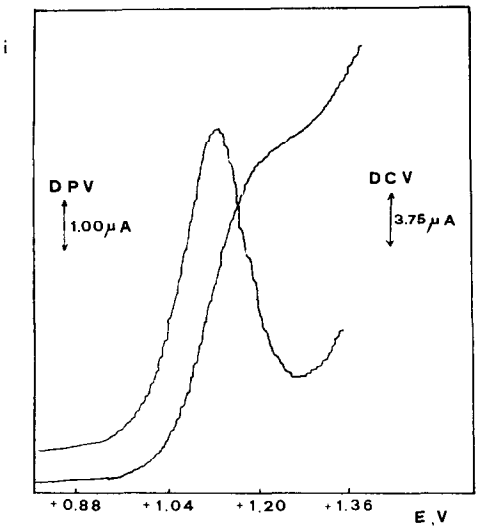

Figure 1
Voltammetric behaviour of vitamin D_3 at a glassy carbon electrode; $[D_3] = 1.3 \times 10^{-4}$ M, $[LiClO_4] = 0.075$ M in methanol.

In previous assays, it was observed that continuous connection of the electrode or successive potential sweeps gave rise to a gradual decrease in the voltammetric signal; the response recovered partially after applying more negative potentials to the electrode before performing new sweeps. To study the reproducibility of the voltammetric signal, several solutions of vitamin D_3 (1.3×10^{-4} M) were prepared; the corresponding voltagrams (both in DC and DP) were recorded using different potential sweep rates ranging from 20 to 1.3 mV s^{-1}. As the sweep rate decreased, which implies a longer application time of positive potential to the working electrode, a considerable decrease also took place in the magnitude of the voltammetric signal (Fig. 2). The most likely cause of such a decrease in the limiting intensities (DC) and peak (DP) is adsorption of vitamin D_3 on the electrode surface. For potential sweep rates greater than 10 mV s^{-1} a reproducible signal was obtained after several voltammetric readings; because of these results, the sweep rate employed in the rest of the work was 20 mV s^{-1}.

The influence of rotation rate was studied since in direct current voltammetry at a solid electrode the limiting intensity, when the process is diffusion-controlled, is governed by the Levich equation (7) according to which i_l is a linear function of $w^{1/2}$ in the 3–30 rps range where w is the rotation rate. From the study conducted on the influence of $w^{1/2}$ on the value of i_l, it was deduced that there was linearity between both variables in the rotation rate range examined (60–2000 r.p.m.); thus, the controlling process may be the diffusion of vitamin D_3 towards the glassy carbon electrode. The rotation rate used in the rest of the studies was 1580 r.p.m. since this rate produces a sufficiently sensitive signal.

With DP voltamperometry, the magnitude of the pulse applied affects the peak intensity, in this work in a practically linear fashion; however, a pulse amplitude of +50 mV was chosen because the response was sensitive and the peak resolution was better when greater ΔE values were employed.

The influence of temperature on the voltammetric process was studied to check the occurrence of adsorption of vitamin D_3 on the electrode surface. Voltammetric readings were performed with DC and DP voltammetry of 1.3×10^{-4} M vitamin D_3 in the medium at 10–50°C. The temperature coefficients corresponding to the DC technique were lower than those of a pure diffusion process; it appears that adsorption could be involved, at least partially, in the overall electrochemical process.

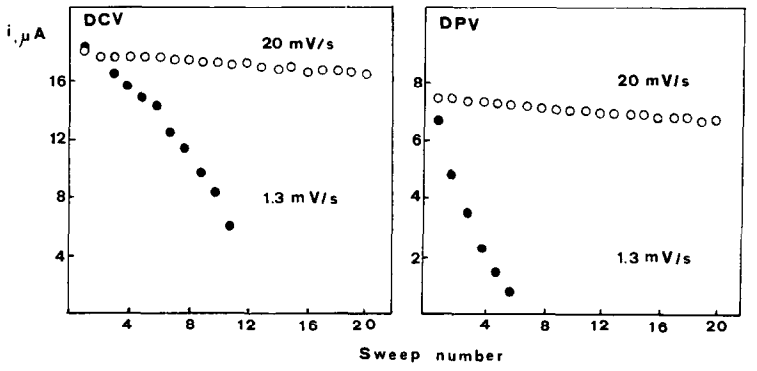

Figure 2
Study of the reproducibility of the voltammetric signal with the potential sweep rate; $[D_3] = 1.3 \times 10^{-4}$ M, $[LiClO_4] = 0.075$ M in methanol.

Study of the reversibility of the system was carried out by performing a logarithmic analysis of the voltagrams obtained by the DC technique and application of the criteria $\Delta E = E^a - E^c$ and $i_p{}^a/i_p{}^c = 1$ using the DP technique, from the results it is deduced that the process is irreversible.

The influence of the concentration of vitamin D_3 on the limiting intensity (DC) and peak intensity (DP) was studied by reading the corresponding voltagrams of different solutions of vitamin D_3 in the 2.1×10^{-6}–7.8×10^{-4} M concentration range. For concentrations lower than 4×10^{-4} M good linearity is seen between i_l or i_p and the concentration of vitamin D_3

$$i_l(\mu A) = 0.104 + 1.53 \times 10^5 C(M); \; r = 0.999.$$

$$i_p(\mu A) = 0.033 + 8.6 \times 10^4 C(M); \; r = 0.998.$$

The absence of linearity between both variables for vitamin D_3 concentrations above 4×10^{-4} M points to saturation of the electrode, which confirms adsorption of the substance on to the electrode surface.

Determination of vitamin D_3

In view of the linearity between the limiting and peak intensities and the concentration of vitamin D_3 the following procedure is proposed for the determination of the compound. Solutions of vitamin D_3 (2×10^{-6}–4×10^{-4} M) are prepared, using methanol as solvent and 0.075 M $LiClO_4$ as the supporting electrolyte. Each solution is placed in the electrolysis cell and the corresponding voltagrams are recorded by potential sweeps in the +0.8–+1.8 V (versus SCE) range at a rate of 30 mV s^{-1} with a rotation rate of the glassy carbon electrode of 1580 r.p.m.; the wave appearing at +1.1 V is evaluated. When the DP technique is employed, the pulse applied is +50 mV and a well-defined peak appears at the same potential.

The detection limit for both techniques was calculated according to the Kaiser criterion; this limit was 1.49×10^{-6} M with DC voltammetry and 2.15×10^{-5} M when the DP technique was used. These values confirm that the use of solid electrodes does not improve the detection limit when differential pulse voltammetry is employed. Likewise, the precision of the method was determined by using the procedure to obtain

Table 1
Determination of vitamin D_3 in pharmaceutical formulations (DC voltammetry)

Sample	Content*	Found mg
Vitamin D_3 Fuerte Bayer	15	14.2
	15	15.9
	15	14.3
	15	13.7
	15	16.2†
Vitaendil	5	4.8
	5	4.8
	5	5.8†

*Content according to the manufacturer's laboratory.
†By the standard addition method.

the voltammetric recordings corresponding to 10 solutions containing 5.28×10^{-5} M vitamin D_3. The relative standard deviations obtained were 1.13% and 2.6% using the direct current and differential pulse techniques, respectively.

Determination of vitamin D_3 in pharmaceutical preparations

The proposed electroanalytical method was applied to the determination of the vitamin D_3 content in pharmaceutical preparations. The analytical findings obtained (Table 1) are in agreement with those provided by the manufacturer's laboratories both when carrying out the determination by use of a calibration method and when using the standard addition method.

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