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Carbon Surfaces for the Oxidative Quantification of Pravastatin: Glassy-Carbon *vs.* Screen-Printed Carbon Electrodes

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ABSTRACT

The electrooxidative behavior of pravastatin (PRV) in aqueous media was studied by square-wave voltammetry at a glassy-carbon electrode (GCE) and at a screen-printed carbon electrode (SPCE). Maximum peak current intensities in a pH 5.0 buffer were obtained at +1.3 V *vs.* AgCl/Ag and +1.0 V *vs.* Ag for the GCE and SPCE surface respectively. Validation of the developed methodologies revealed good performance characteristics and confirmed their applicability to the quantification of PRV in pharmaceutical products, without significant sample pretreatment. A comparative analysis between the two electrode types showed that SPCEs are preferred as an electrode surface because of their higher sensitivity and the elimination of the need to clean the electrode's surface for its renewal, which frequently is, if not always, the rate-limiting step in voltammetric analysis.

Key words: pravastatin, square-wave voltammetry, electrochemical oxidation, glassy-carbon electrode, screen-printed carbon electrode

INTRODUCTION

Lipid-modifying interventions have been shown to decrease the risk of coronary heart disease both in patients with hypercholesterolaemia and in those with relatively normal levels of low-density lipoprotein cholesterol[1].

Pravastatin (Figure 1) belongs to the class of the most widely used lipid-lowering drugs, statins. It is a water-soluble cholesterol-reducing agent, which acts by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. The enzyme catalyses the conversion of HMG-CoA to mevalonate, the rate-limiting step in cholesterol synthesis[2]. Competitive inhibition of this enzyme by the statins decreases hepatocyte cholesterol synthesis[1].

A large number of high-performance liquid chromatographic (HPLC) procedures have been developed for PRV determination in a variety of matrices, such as pharmaceuticals, serum, plasma, urine and sewage. The most recently used methods are based on HPLC coupled with ultraviolet/diode array[3-7] and mass[5,8-15] detectors. Recently, a visible spectrophotometric method has also been described[16].

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Prior to the present work, there was only one electroanalytical study on PRV that had been published[17]. The study focused on the reductive and adsorptive behaviours of PRV at a hanging mercury-drop electrode. No study on the oxidative behaviour of PRV has been published to date.

Within the field of electroanalytical science, various kinds of disposable electrochemical sensors based on SPCEs are becoming increasingly important, as they help in the development of on-site monitoring for clinical, environmental, biological, food and industrial analysis.

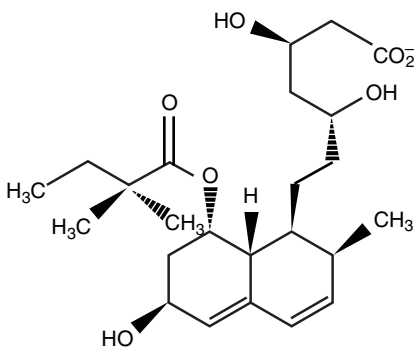

Figure 1. Chemical structure of pravastatin.

Features such as design flexibility, good reproducibility, low reagent consumption and particularly the possibility of mass production, make them an attractive alternative for sensor building materials[18].

In the present work, the oxidation of PRV at a glassy-carbon electrode (GCE) and at a screen-printed carbon electrode (SPCE) was studied with the use of square-wave voltammetry (SWV). Two different methods were developed and successfully applied to the quantification of PRV in a pharmaceutical product of current therapeutic use.

MATERIALS AND METHODS

Pravastatin sodium standard was purchased from Sigma-Aldrich and used without further purification. The standard was stored at 4°C. Stock solutions were obtained by dissolving adequately weighed amounts of the standard in deionised water (conductivity < 0.1 μS cm^{-1}). To obtain the desired concentration for analysis, the stock solutions were diluted with electrolyte solution [Britton-Robinson buffer (I = 0.3 mol/L), pH 5.0]. All other chemicals were of analytical grade.

Voltammetric measurements were performed with an Autolab PGSTAT12 potentiostat (Metrohm-EcoChemie, Utrecht, The Netherlands), controlled by a computer installed with GPES 4.9 software (Metrohm-EcoChemie Utrecht, The Netherlands). When using the GCE, the potentiostat was coupled with a Metrohm 663 VA stand containing a three-electrode cell (Metrohm). The cell consisted of a glassy-carbon working electrode (d = 2 mm), an Ag/AgCl/KCl (3 mol/L) reference electrode and a glassy-carbon auxiliary electrode. Commercially available SPCEs (ref. 110 - DropSens, Oviedo, Spain) were also used. These electrodes consisted of a carbon working disk electrode (d = 4 mm), an auxiliary carbon electrode and a silver pseudo-reference electrode, all printed on the same alumina strip. An insulating area served to delimit the working area and electrical contacts. With these electrodes, the experimental set-up was similar to that mentioned above, replacing the electrode-cell with an adaptor for the SPCEs (DSC - DropSens, Oviedo, Spain).

Between measurements, SPCEs were replaced while the surface of the GCE had to be cleaned. The cleaning procedure involved polishing the electrode surface on an abrasive cloth with Al_2O_3 (0.3 mm, BDH chemicals). The electrodes were then rinsed with deionised water and dried.

For assays using the GCE, the background voltammogram was obtained by introducing 10.00 mL or 15.00 mL of supporting electrolyte into a voltammetric cell. Before analysis, deoxygenation was performed by purging the electrolyte with oxygen-free nitrogen for 300 s, with stirring. After the deoxygenation process, the stirring was stopped and the background voltammogram was recorded. The electrode was then cleaned and the required amount of PRV standard solution was added to the cell. The procedure was repeated using a 10-s nitrogen-purge between measurements. For SPCEs, voltammetric measurements were performed by placing a 50-μL drop of the analyte solution over the exposed area. To record the background signal, the analyte solution was replaced with the electrolyte. In both cases, the square-wave voltammograms were obtained by applying a potential scan in the positive direction.

To evaluate the precision of using the GCE for the analysis of PRV in terms of repeatability and intermediate precision, PRV solutions of 8.0×10^{-5}, 1.0×10^{-4} and 1.2×10^{-4} mol/L were analysed five times per day over three consecutive days. For the SPCE, the same approach was used by analysing PRV solutions of 1.0×10^{-4}, 3.0×10^{-4} and 7.5×10^{-4} mol/L.

In order to evaluate the selectivity and accuracy of the procedure for the analysis of PRV in Pravastatin Alter (Alter S.A., 10 mg PRV/tablet, gross weight = 0.1 g), five tablets were weighed and finely powdered. About 0.108 g of the resulting mixture was transferred to a 10.00-mL volumetric flask and water was added. The flask was then placed in an ultrasonic bath for 15 min to extract PRV from the matrix. For the method using the GCE, a 442.5-μL aliquot of the resulting suspension was placed in 10.00 mL of supporting electrolyte contained in the voltammetric cell and analysed. Subsequently, three standard additions were made (5.0×10^{-5}, 1.00×10^{-4} and 1.50×10^{-4} mol/L) and the voltammetric scan was applied after each addition. Selectivity was evaluated by recovery studies and comparing the slope of an external standard calibration curve with the slope of the standard addition curve. For the method using the SPCE, five 105.9-μL aliquots of the suspension were placed in five 5.00-mL volumetric flasks and standard additions of 0, 5.00×10^{-5}, 2.50×10^{-4}, 4.50×10^{-4} and 7.00×10^{-4} mol/L were made. The resulting solutions were then analysed by the developed SWV procedure. Selectivity was evaluated by comparing the slope of an external standard calibration curve with the slope of the standard addition curve.

For the quantification of PRV in Pravastatin Alter, five tablets were weighed and finely powdered. About 0.108 g of the resulting mixture was transferred to 50.00-mL (GCE) or 10.00-mL (SPCE) volumetric flasks and placed in an ultrasonic bath for 15 min in order to extract PRV from the matrix. The resulting suspension was then diluted in the voltammetric cell (GCE) or in 5.00-mL volumetric flasks (SPCE) with supporting electrolyte to obtain the desired PRV concentrations (within the linear ranges) for analysis. Quantification of PRV was carried out by the external calibration method.

RESULTS AND DISCUSSION

I. Optimization

The optimization of the experimental conditions was

performed using the glassy-carbon electrode.

High-frequency square-wave voltammetry was used to study the electrochemical oxidation of PRV at the glassy-carbon electrode. The electrochemical behavior of PRV (1.0×10^{-4} mol/L) was verified over the pH range 1.40 - 11.70, using Britton-Robinson buffers as supporting electrolytes. The results showed that PRV gave rise to one anodic peak over the whole pH range investigated. At a pH value of 5.0, the highest and best-defined oxidation peak was obtained at approximately +1.3 V *vs*. AgCl/Ag.

Since SWV was used for the determination of PRV in a pharmaceutical dosage form, it was necessary to determine the most adequate SWV parameters in order to obtain the best peak characteristics for PRV analysis. The frequency (f), pulse step (ΔE_s) and pulse amplitude (ΔE_p) were optimized using a 1.0×10^{-4} mol/L PRV solution in a Britton-Robinson buffer (pH = 5.0). The influence of f was studied over a range of 10-100 Hz. The peak current intensity (i_p) increased linearly until 90 Hz ($i_p = 0.016 \times f + 0.86$; i_p - μA, f - Hz). A less significant increase of i_p was observed after this value. Hence, an f of 90 Hz was adopted in subsequent experiments. Variation of ΔE_s from 1 to 10 mV and of ΔE_p from 5 to 50 mV resulted in a marked increased of i_p up to 5.25 mV ($i_p = 0.53 \times \Delta E_s + 1.3$; i_p - μA, ΔE_s - mV) and 40 mV ($i_p = 0.12 \times \Delta E_p + 0.52$; i_p - μA, ΔE_p - mV) respectively, after which the i_p values remained constant. These optimized conditions were used in all the subsequent studies.

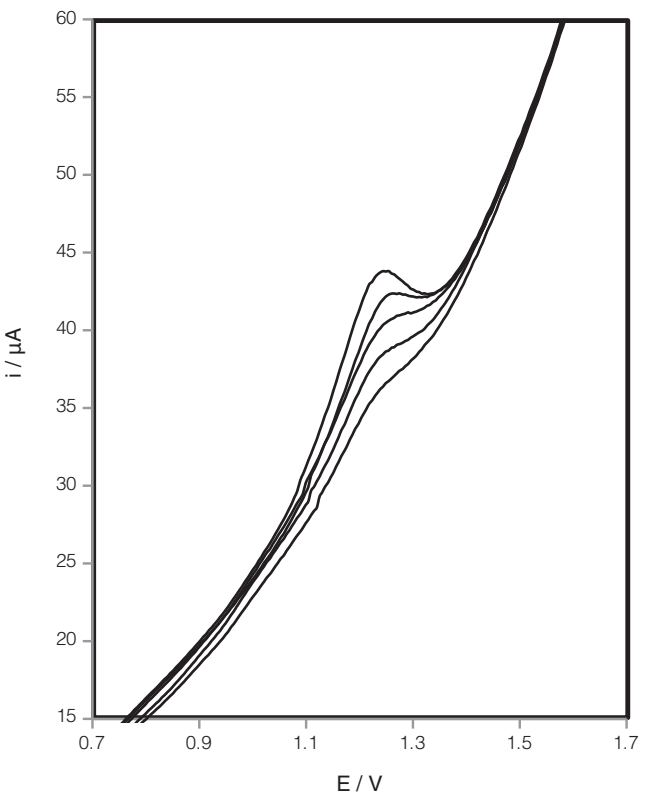

Figure 2. Square-wave voltammograms of PRV in the linear range by GCE. [PRV] (× 10^{-4} mol/L): 1.00, 1.50, 2.00, 2.50 and 3.00 (f: 90 Hz; ΔE_s: 5.25 mV; ΔE_p: 40 mV). (i/μA: current intensity/micro-ampere; E/V: applied potential/volt).

II. *Validation*

(I) *Glassy-Carbon Electrode*

A linear relationship between peak current intensity and PRV concentration was obtained for the interval between 6.0×10^{-5} and 9.2×10^{-4} mol/L. Typical voltammograms of PRV concentrations within this range are shown in Figure 2.

Limits of detection (LOD) and quantification (LOQ) [19] as well as other calibration plot characteristics are given in Table 1. The results of the precision studies (Table 2) confirmed that the method was precise.

To verify whether excipients in the Pravastatin Alter tablets interfered with the analysis, recovery and selectivity studies were performed. Recovery values between 97.9% and 102.3% were obtained, confirming that the method was accurate and that excipients did not interfere significantly with the analysis. In addition, the ratio between the slope of the external standard calibration

Table 1. Analytical data from the calibration plot in the determination of PRV by SWV

Property	GCE	SPCE
Linear range (μmol/L)	60 - 920	50 - 1000
Correlation coefficient	0.999 (n = 8)	0.999 (n = 6)
Slope (mA L mol^{-1})	5.43	82
Standard error slope (mA L mol^{-1})	0.04	1.3
Intercept (μA)	0.20	8.7
Standard error intercept (μA)	0.02	0.8
LOD (μmol/L)	11	30
LOQ (μmol/L)	37	101

Table 2. Results from the evaluation of precision*

Electrode	Repeatability** (RSD %, n = 5)	Intermediate Precision** (RSD %, n = 3)
GCE	1.4 (0.8)	1.0 (0.8)
	0.9 (1.0)	3.8 (1.0)
	5.3 (1.2)	3.0 (1.2)
SPCE	4.2 (1.0)	3.5 (1.0)
	4.4 (3.0)	6.1 (3.0)
	1.3 (7.5)	0.6 (7.5)

* Concentrations in 10^{-4} mol/L are given in parentheses.
** RSD: Relative standard deviation

plot and the slope of a standard addition curve was found to be 1.1, indicating that the method was selective for the determination of PRV in this sample.

(II) *Screen-Printed Carbon Electrodes*

With the previously optimized SWV parameters using the glassy-carbon electrode, the oxidation peak of PRV was obtained at about +1.0 V *vs.* Ag. Using the previously optimized SWV parameters and analytical conditions, validation of the SWV procedure for PRV analysis was repeated using SPCEs. Table 1 lists the characteristics of the calibration curve and the respective LOD and LOQ values calculated using the curve. Typical voltammograms of PRV concentrations within the linear range are shown in Figure 3.

The results from the evaluation of precision are reported in Table 2, indicating that the method was precise. A 1.1 ratio between the slope of an external standard calibration plot and the slope of a standard addition curve confirmed the method's selectivity.

III. *Application*

In the application of the SWV method to the quantification of PRV in the pharmaceutical product, Pravastatin Alter, the labeled values (10 mg per tablet) were in good agreement with the obtained results: 9.97 ± 1.3 mg/tablet (n = 3) using the SPCE and 9.78 ± 0.84 mg/tablet (n = 3) using the GCE.

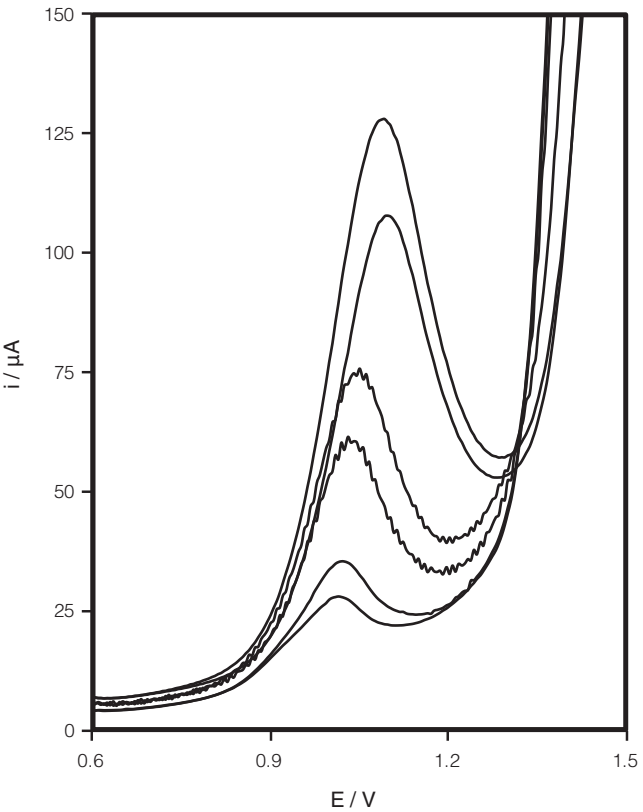

Figure 3. Square-wave voltammograms of PRV in the linear range by SPCE.
[PRV] (× 10^{-4} mol/L): 0.500, 1.00, 3.00, 5.00, 7.00 and 10.0 (f : 90 Hz; ΔE_s: 5.25 mV; ΔE_p: 40 mV). (i/μA: current intensity/microampere; E/V: applied potential/volt).

CONCLUSIONS

The present study showed that both electrode types can be successfully applied to the quantification of PRV in a pharmaceutical product. No significant differences were found in terms of detection limits, precision and accuracy. Both methods provided simple, sensitive, accurate, selective, fast and low cost quantifications of PRV in pharmaceutical dosage forms.

Besides the better sensitivity of the SPCE, which is probably due to the difference in the active area of the electrode, SPCEs have another important advantage over the GCE - The use of SPCEs eliminated the need to clean the electrode's surface for its renewal, which frequently is, if not always, the rate-limiting step in voltammetric analysis.

When compared with published non-chromatographic methods for pravastatin analysis in pharmaceuticals, the obtained linear ranges and limits of detection were in the same order of magnitude. Although the limits of detection of the developed methods were 2 to 4 orders of magnitude higher than those obtained with most of the published chromatographic methods, they were sufficiently low for the proposed application.

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