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Determination Antidiabetic Drugs of Pioglitazone Based on Silver Electrodeusing in a Flow through a Voltammetric Sensor.

Lai-Hao Wang *, and Yan-Lin Song.

Department of Medical Chemistry, Chia Nan University of Pharmacy and Science, 60 Erh-Jen Road, Section 1, Jen Te, Tainan 71743, Taiwan.

ABSTRACT

We develop a method that uses electrochemical impedance spectroscopy (EIS) to quantitatively determine pioglitazone, a thiazolidine-2, 4-diones derivative. The method is using a silver electrode. An increase in the pioglitazone concentration results in an increase in the Faradaic electron-transfer resistance (R_{et}) obtained from the EIS measurements. Pioglitazone is quantified from the linear variation of the sensor response (R_{et}) as a function of the pioglitazone concentration in solution. The method is straightforward and nondestructive. The dynamic range for determining pioglitazone is extended by more than two orders of magnitude. The method can be applied to the quantitatively determining pioglitazone in antidiabetic drugs. Findings using EIS and high-performance liquid chromatography with a flow-through voltammetric detector and ultraviolet detector are comparable.

Keywords: Electrochemical impedance spectroscopy; voltammetric sensor; pioglitazone in antidiabetic drugs.

Corresponding author

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INTRODUCTION

Thiazolidine-2, 4-diones (TZDs) are a class of antidiabetic compounds that are derived from the antilipidemic and weakly antihyperglycemic agents. The delivery of pioglitazone, which has the chemical name (±)-5-[[4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl]methyl]-2,4-] thiazolidinedione**,** is one of the potent compounds of TZDs used in clinic. Therapies directly to the site would ultimately allow higher concentrations of the drug to be delivered and prolong circulation time in vivo to enhance the therapeutic outcome of the drug [1-6]. Several analytical methods for thiazolidine derivatives have appeared in the literature [7-16]. In general, they involve liquid chromatography with ultraviolet and mass detection [7-9] and electrochemical detection [10-13]. Hoverer, but not all of them are suitable for the routine detection and these methods require sample pretreatment. The electrochemical oxidation of the biological compounds of rosiglitazone in acid solution on a glassy carbon electrode has been studied by D.T. Burcu [14]. W. A. Badawy studied the voltammetric determination of thiazolidine derivatives such as rosiglitazone and pioglitazone on carbon paste (CPE) and glassy carbon (GCE) electrodes in Briton-Robinson buffer solution has been studied by [15]. Ali F. Al-Ghamdi studied the electrochemical determination of rosiglitazone on a hanging mercury drop electrode [16]. Electrochemical impedance spectroscopy (EIS) is a powerful tool for examining many chemical and physical processes in solutions as well as solids [17-19]. Among all the detection methods, electrochemical impedance spectroscopy (EIS) has raised great interest because of its high sensitivity and label free characteristics, which are uniquely attractive for *in vivo* diagnostics and biosensors [20-31]. Furthermore, there have been no reports in the literature concerning the determination of pharmaceutical dosage of pioglitazone when using EIS.

This study investigates, the electrochemical oxidation of pioglitazone using a gold and silver electrode has been investigated by impedance and cyclic voltammetry (CV). We also design electrochemical flow cell devices used for studying pioglitazone flow through silver electrode electrochemical processes. The optimum experimental conditions for the determination of pioglitazone in pharmaceutical products are described in this paper.

EXPERIMENTAL

Apparatus and Materials

All electrochemical measurements were performed with a potentiostat–galvanostat (SP-150; Bio-Logic SAS, 1 rue de l'Europe 38640 - CLAIX - FRANCE) with a conventional three-electrode configuration with gold and silver as working electrodes. Potentials were measured versus the Ag/AgCl electrode (RE-1; Bioanalytical Systems, West Lafayette, IN, USA), and a platinum wire was used as the auxiliary electrode. A high-performance liquid chromatography (HPLC) system(LC-10 ADvp; Shimadzu, Kyoto, Japan) containing a Rheodine 7125 injection valve with a 20 mL sample loop was coupled to an amperometric detector (Decade SDC; Antec Leyden B.V., Zoeterwoude, Netherlands). The flow cell was designed with the following electrodes: an Ag/AgCl/0.1 M KCl reference electrode (Bioanalytical Systems), a stainless steel

auxiliary electrode, and a silver electrode (length: 8 cm; i.d., 3 mm) as the working electrode for detecting pioglitazone. Pioglitazone (Scheme 1) was purchased from Sigma (St. Louis, MO, USA). The antidiabetic drugs investigated were purchased at a local department store. All other reagents were locally purchased and were of analytical grade.

Determining pioglitazone in antidiabetic drugs by CV and EIS

Cyclic voltammetry (CV) and EIS were performed in a phosphate buffer (pH 2.41 and 6.56), Britton and Robison buffer (pH 2.61-4.71), and lithium perchlorate ($LiClO_4$) solutions as supporting electrolytes on a silver electrode. CV potentials ranged from 0.6V to +0.5 V at a scan rate of 25 mV s^{-1}. The EIS and CV data acquisition were performed using SP-150, Bio-Logic SAS and EC-Lab® softwares. The impedance spectra were recorded over a frequency range of 0.01 Hz to 100 kHz, using a sinusoidal excitation signal, superimposed on a *dc* potential of + 0.2 V. Excitation amplitude of 10 mV was used throughout.

Stock solution of standard was prepared by dissolving the appropriate amount of pioglitazone in methanol. A set of standard solutions was produced by diluting aliquots of the stock solutions with methanol to 10 mL in calibrated flasks. Taking into account the pioglitazone content of the antidiabetic drugs samples (approx. 0.05-0.1 g) the latter were weighed accurately in a 15 mL beaker, diluted to about 10 mL methanol dissolved and centrifuged. The supernatant was transferred into a 5 mL calibrated flasks. An aliquot of the solution was filtered through a 0.45 µm membrane filter prior to HPLC analysis. We used a simple dilution process for the EIS and CV experiments as well as for the standard solution.

Determining pioglitazone in antidiabetic drugs using a flow-through voltammetric detector

A flow-through electrolysis cell was used for DC-mode electrochemical detection. The detection cell was constructed in our laboratory. Reversed phase HPLC was done on a Hypersil C_8 (250 mm x 4.6 mm) column eluted with various methanol–water (40: 60, v/v), (50: 50, v/v) and (60: 40, v/v) containing1.0 mM of KH_2PO_4 (pH 3.85) as the mobile phase, at a flow rate of 1.0 mL min^{-1}. It was examined using an ultraviolet (UV) detector set at 223 nm. The electrochemical detector was operated at + 0.2 V to -0.6 V. A chromatograph was obtained using 20 mL of the prepared standard solution under the operating conditions described above.

RESULTS AND DISCUSSION

Voltammetric behavior of pioglitazone on Au and Ag electrodes

Cyclic voltammograms (CV) of pioglitazone on Au and Ag electrodes show potentials ranging between 0 and 1.5 V for Au, and between - 0.6 V and 0.5 V for Ag (Fig. 1). We observe one wave of pioglitazone (62 mg L^{-1}) and its stripping at 0.255 V and one cathodic peak at – 0.038 V on Ag. It was found that Ag exhibits a pronounced electrocatalytical effect (Fig. 1). The cyclic voltammetric peak potential and current for pioglitazone at the Ag electrode were 0.255 V and 123 µA, respectively. However, the peak potential was not obvious at Au electrode.

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Therefore, we chose to use the Ag electrode to determine the concentrations of pioglitazone in antidiabetic drugs.

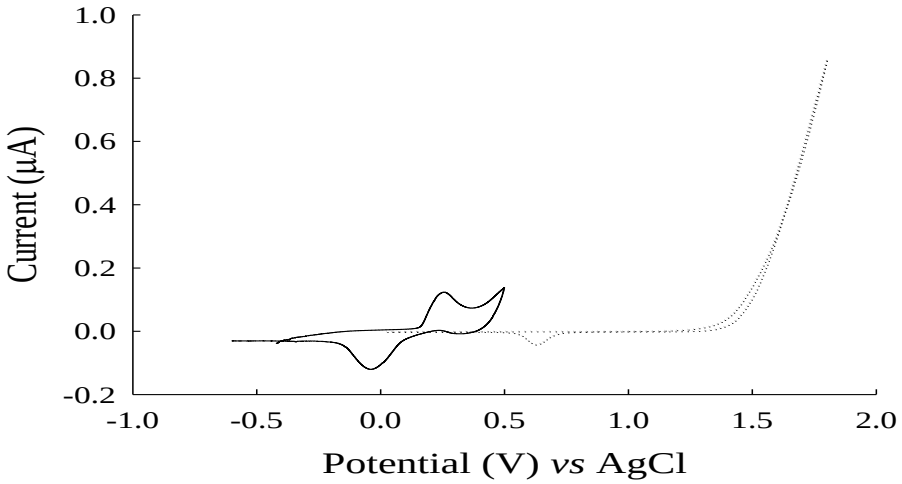

Figure 1: Cyclic voltammograms of pioglitazone (62 mg L^{-1}) in Britton and Robison buffer (pH 3.90). The solid line is for a Ag electrode (peak at 0.255 V, 123 μA); the dotted line is for a Au electrode (not determined).

Effect of solution pH

A Nyquist diagram of various supporting electrolytes with the development of the Ag electrode showed similar linear portions for each buffer solution (Fig.2A)—BR buffer (pH 2.16), lithium perchlorate(pH 6.52), phosphate buffer(pH 6.56) and BR buffer(pH 3.90)—which indicates that these supporting electrolytes improve the electron transfer rate between the electrode and solution. Furthermore, the electrode in BR buffer (pH 3.90) generated a decrease in impedance because of the change in the electrical characteristics of the electrode/electrolyte interface. The peak potential and peak current closely depend on the pH of the buffer solution. CV was used to clarify the electrochemical properties of the supporting electrolytes shown in Fig. 2B. The peak current of pioglitazone in the BR buffer (pH 3.90) was higher than that in the other supporting electrolytes. For analytical purposes, the best supporting electrolyte for determining pioglitazone is the Britton-Robinson buffer pH 2.16-3.90: it is more sensitive than the others in this range. BR buffer (pH 3.90) which was selected as the optimal pH valve for determination of pioglitazone.

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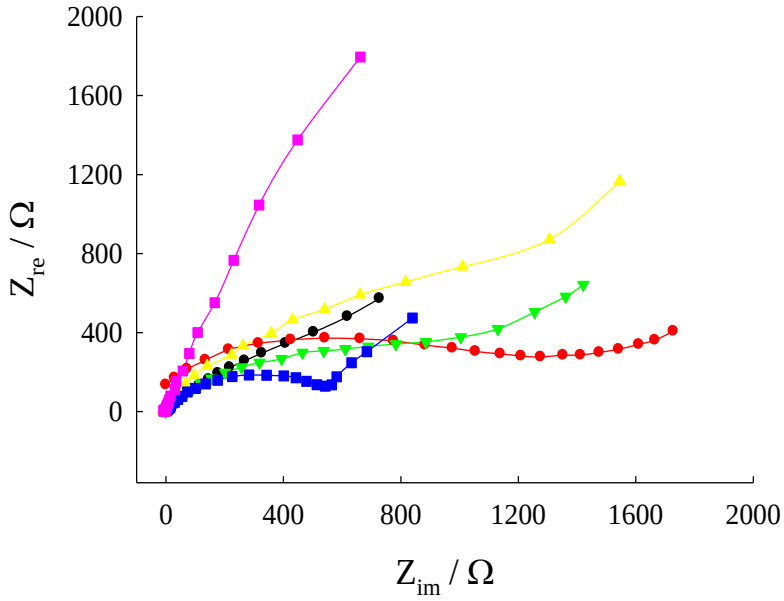

Figure 2A: Nyquist impedance spectra of a Ag electrode in several supporting electrolytes containing pioglitazone(62 mg L^{-1}). The impedance spectra were measured at: phosphate buffer, pH 2.41 (black circle); phosphate buffer, pH 6.56, red circle; lithium perchlorate, pH 6.52 (green triangle down); BR buffer, pH 2.16, (yellow triangle up); BR buffer, pH 3.90 (blue square); BR buffer, pH 4.71(pink square) solutions as supporting electrolytes.

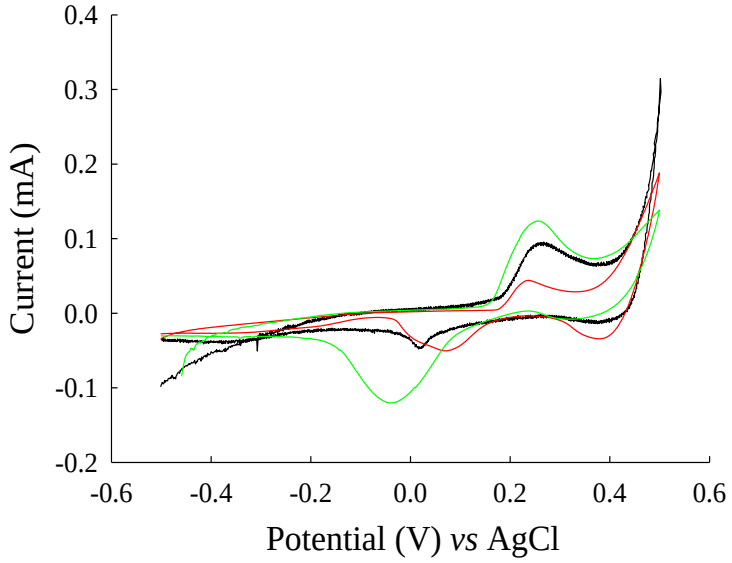

Figure 2B: Cyclic voltammetry (CV) curves of a Ag electrode in several supporting electrolytes containing pioglitazone (62 mg L^{-1}). The CV were measured for BR buffer, pH 3.9 (green line), lithium perchlorate (black line) and BR buffer, pH 2.16 (red line) solutions as supporting electrolytes.

ISSN: 0975-8585

Quantification and sensitivity of EIS

The dynamic range is the ratio of the largest target concentration and the limit of detection. The sensitivity of Ag electrode was investigated by measuring the changes in R_{et} under various concentrations of pioglitazone. The EIS was obtained using the standard addition method. The electron transfer impedance R_{et} increased with the concentration of pioglitazone (Fig. 3A and3 B). R_{et} has a good linear relationship with pioglitazone concentration c in the range of 2 – 62 mg L^{-1} with the linear equation R_{et} = 7.37 x + 37.0(unit of C, mg L^{-1}), and linear regression coefficient (r) of 0.9906. This observation is consistent of what we have seen in the previous EIS examples, where the R_{et} has generally increases with concentrations. The analytical performance of the pioglitazone is compared with that of the conventional HPLC methods. It is found that the detection limit (0.05 mg L^{-1}) of the Ag electrode developed is comparable or even lower. The results are in good agreement. A representative EIS of a commercial antidiabetic drug is shown in Fig. 4A and 4B.

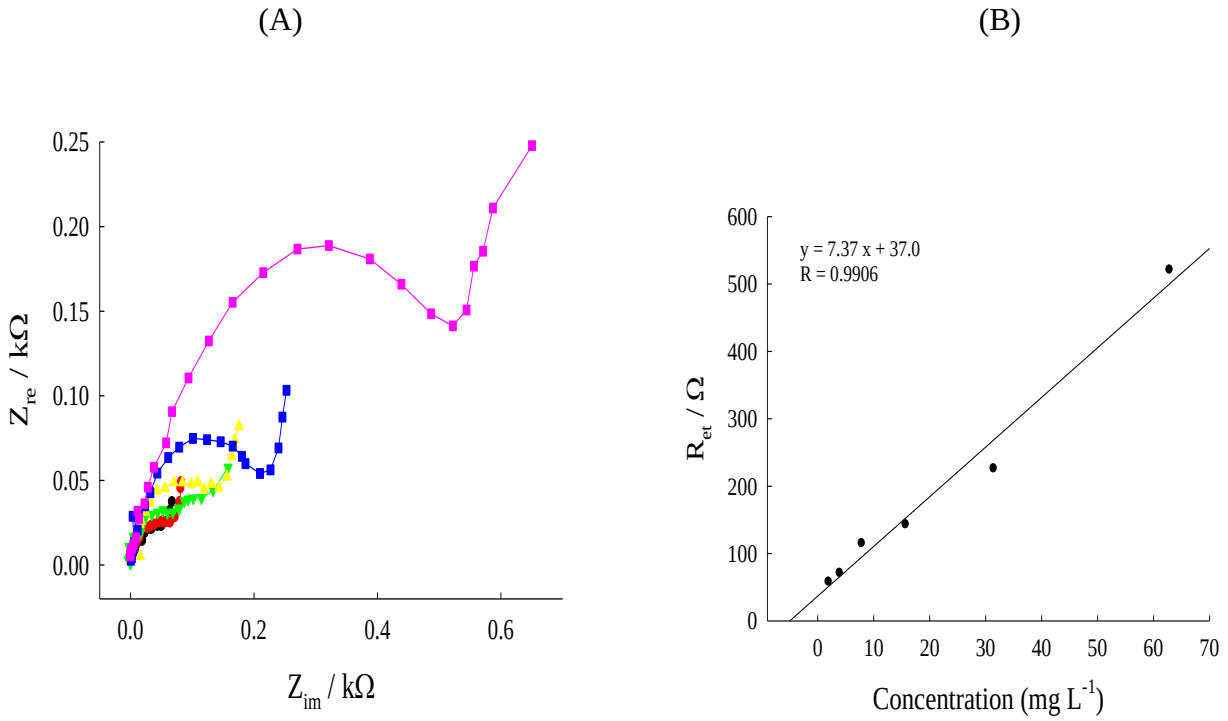

Figure 3: (A) Nyquist plots of impedance spectra obtained on Ag electrode for different concentrations of pioglitazone in Britton and Robison buffer (pH 3.90) (B) The calibration curve obtained using R_{ct} as a function of pioglitazone concentration at E_{DC} = 0.2 V *vs.* Ag/AgCl, linear regression equation: Y (Ω) = 7.37 x + 37 (mg L^{-1}), R = 0.9906.

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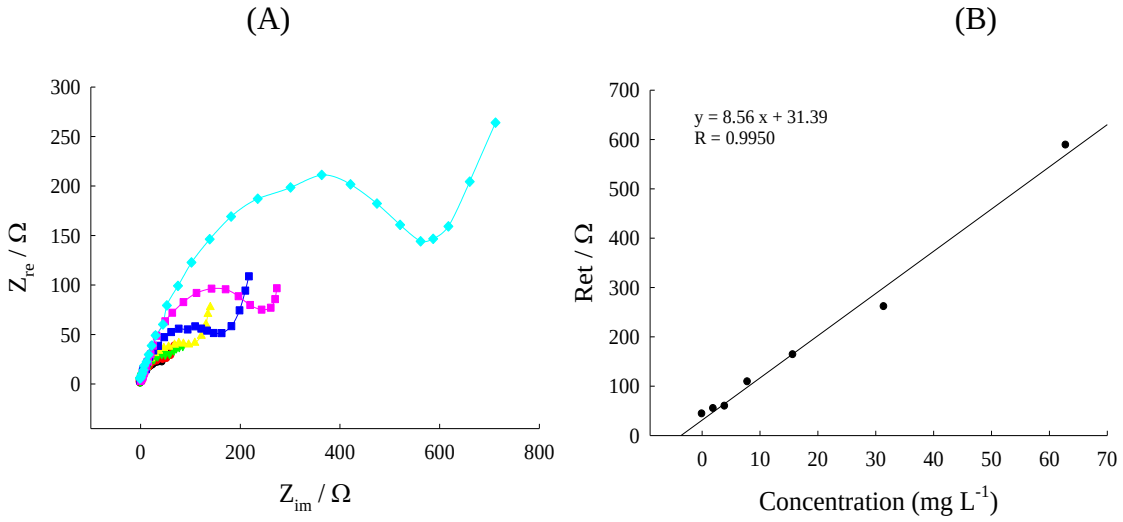


Figure 4 (A) Nyquist plots of impedance spectra obtained from commercial antidiabetic drug after they had been spiked with pioglitazone solutions of 0 mg L^{-1} (black circle), 2 mg L^{-1} (red triangle), 4 mg L^{-1} (green triangle down), 8 mg L^{-1} (yellow triangle up), 16 mg L^{-1} (blue square), 32 mg L^{-1} (pink square), 64 mg L^{-1} (cyan diamond); (B) The calibration plot.

Application to a flow-through voltammetric detector

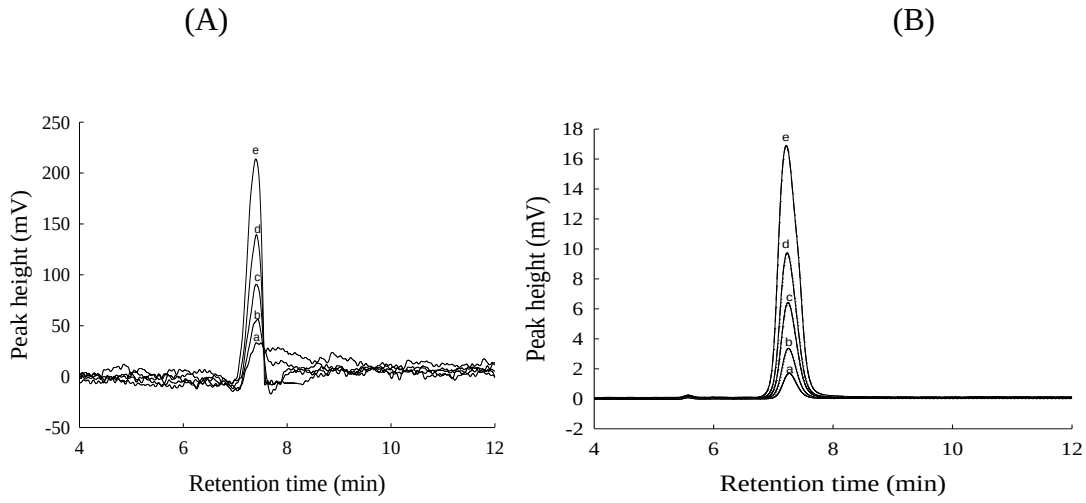


Fig. 5 Calibration graphs for pioglitazone using (A) LC-ECD and (B) LC-UV.
Stationary phase: Hypersil C_8 (250 mm x 4.6 mm) column; mobile phase: methanol–water (60:40, v/v) containing 0.1 mM of K2HPO4 (pH 3.85); flow rate: 1.0 mL min^{-1}.Electrode potential was -0.6 V vs. the Ag/AgCl reference electrode; the ultraviolet detector was set at 223 nm.

The voltammetric detector was operated at −0.6 V. Using the injection valve, 20 µL of the prepared standard solution was chromatographed under the operating conditions described above. The lower limit of quantitative detection in our method was approximately 0.8 µg and linear to 40 mg L^{-1}. The calibration graph plots obtained by plotting the peak area

against the concentration of pioglitazone show good linearity over the range 0.2- 40 mg L^{-1}. The regression equations were y = 8.08 x + 21.4 (*r* = 0.9919) and y = 1.34 x + 2.23 (*r* = 0.9926) for the LC-ECD and LC-UV wavelengths at 223 nm, respectively (Fig. 5A and 5B). Subsequently, we developed a simple and sensitive green electrochemical procedure for determining pioglitazone. The representative LC-ECD chromatograms for the commercial antidiabetic drug and pioglitazone with retention characteristics at the Ag electrode were 7.3 min (Fig. 6A and 6B).

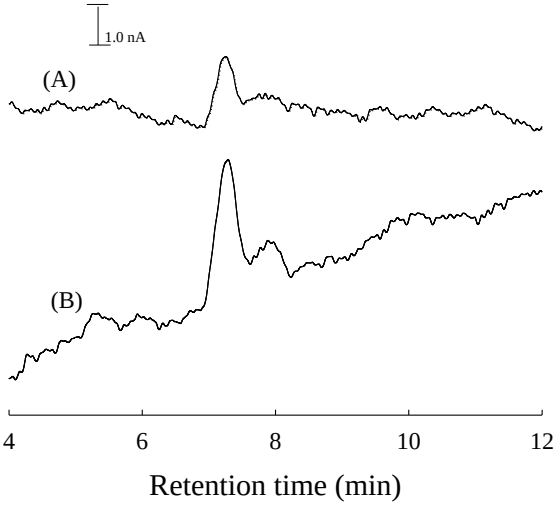
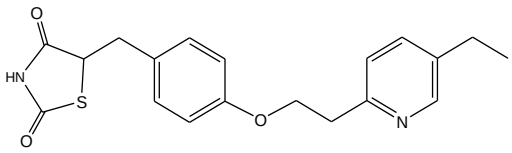


Figure 6: Chromatograms obtained by LC-ECD from commercial antidiabetic drug (A) and (B) pioglitazone standard solution at Ag working electrode. The analytical conditions were identical with those used to obtain Fig. 5A.



Scheme 1 Pioglitazone structure

Analytical applications

In order to test the applicability of the developed Ag electrode, an antidiabetic drug was analyzed using the standard addition method. Recovery tests were carried out on drug products to evaluate the reproducibility and accuracy of the proposed EIS method. The antidiabetic drug sample was diluted with methanol, and the analytes were spiked with different concentrations of pioglitazone. The recoveries ranged from 93% to 102%. Table 1 shows the results (with an average of three measurements) obtained by EIS and LC-ECD and LC-UV (used as the reference method). Good agreement is observed.

ISSN: 0975-8585

Table 1 Comparison of pioglitazone in commercial antidiabetic samples using the Ag electrode by electrochemical impedance spectroscopy (EIS) and high-performance liquid chromatography with voltammetric sensor (LC-ECD) and ultraviolet detector (LC- UV)

Samples	Label (mg)	Concentration (%, w/w) [a]		
		EIS	LC-ECD	LC-UV
Sample 1	30.0/ tablet	25.0 ± 0.64	25.4 ± 1.02	22.6 ± 0.42
Sample 2	15.0/ tablet	21.1± 0.36	22.2 ± 0.37	21.7 ± 0.61
Sample 3	Unknown	8.63 ±0.12	8.46 ± 0.07	7.85 ± 0.07

[a]Number of determinations.
[b]Values indicate standard deviation.

CONCLUSIONS

The EIS measurements of pioglitazone were performed on the Ag electrode. Our data indicate that EIS is faster than LC-ECD for detecting pioglitazone adsorption on electrode surfaces. We have successfully demonstrated that the Ag electrode is a feasible electrode for determining pioglitazone. It exhibits a good analytical performance for the impedance detection of pioglitazone with a low limit of quantitation, rapid response, a satisfactory linear range and good stability and selectively. The presented electrode was successfully used to determine pioglitazone in commercial an antidiabetic drug. The method is simple and easy to performed. Furthermore, the cost of using the presented electrode is lower than that of currently used methods.

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