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Short communication

Periodically interrupted amperometry at membrane coated electrodes: A simplified pulsed amperometry

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Abstract

Amperometric detection combined with separation technique or with selective molecular recognition step can be very effective solving quantitative analytical tasks. When the amperometric working electrode surface needs cleaning or reactivation, pulsed amperometric technique can be the choice. Coating working electrodes with different sensitizing or protecting layer is quite common in the practice of voltammetric analysis. In these studies the behavior of coated electrodes using a simplified pulsed amperometric working program which can be named periodically interrupted amperometric (PIA) detection has been investigated. Rotating platinum, and carbon paste electrodes coated with dialysis film or porcine intestinal membrane were used in the experiments. The signal in case of electrochemical oxidation of hydrogen peroxide and ascorbic acid at convective conditions has been evaluated. The signal, obtained with conventional amperometry has been compared with signal collected with a periodically interrupted amperometric measuring program, allowing time for the diffusion to reload the diffusion layer at the electrode surface. The sensitivity and the lower limit of detection (4.5×10^{-7} M for ascorbic acid and 2×10^{-6} M for H_2O_2) proved superior in case of the periodically interrupted amperometry.

Keywords: Pulsed amperometry; Ascorbic acid; Hydrogen peroxide; Sensitivity; Lower detection limit

1. Introduction

The passivation of the electrode surface is a quite common incident in practice of voltammetry with solid electrodes [1]. Most often mechanical renewing, polishing is used to take off the adsorbed layer. However, the reproducibility of the mechanical renewing is limited, and it is also very inconvenient [2]. It was early noticed, that electrical treatments such as reductive or oxidative potential pulses could regenerate the electrode surface [3]. Even the surface layer formed on the electrode during the potential pulse can act as electrocatalyst [4] in different reactions enhancing the reaction rate.

Since the pioneering work of Kissinger, Adams and others the amperometric detection is common in reverse phase HPLC analysis [5]. However if the product of electrode reaction passivates the electrode or in presence of electrode fouling matrix components continuous amperometric detection cannot be applied. For these cases pulsed amperometric methods have been worked out and were successfully used in HPLC analysis [6,7]. Applying proper pulsed amperometric detection (PAD) measuring program can provide periodical regeneration of the electrocatalytic surface layer of the working electrode resulting in enhanced sensitivity of detection.

Reports appeared about the advantages and applications of different triple or more step wave measuring programs using pulsed amperometric (PAD) [8,9], pulsed coulometric (PCD) [10] or potential sweep pulsed coulometric detection (PS-PCD) [11]. Most of experiments using the PAD or PCD technique are related to HPLC and ion exchange chromatographic detection. The characters of the measuring wave steps – in their case – are selected to accomplish electrode surface regeneration quickly in a reproducible way. In most chromatographic measurements applying electrochemical detection, the flowing eluent is in direct contact with the working electrode surface. Owing to the convective conditions the analyte needs to pass through the very thin diffusion layer on the electrode surface before taking part in the electrode process.

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Besides chromatographic methods, pulsed techniques are also used in other fields of electrochemical research. The same is true for FIA experiments like in the work of Gerlache and coworkers. They developed a new method for H_2O_2 analysis with gold electrode by pulsed amperometry [12], and could successfully measure H_2O_2 down to the micromolar range by using a three-potential-step amperometric measurement.

In case of chemically modified electrodes, however the transport of the analyte into the site of electrode reaction is often hindered. Sometimes enhanced selectivity is provided by coating the electrode surface with a size exclusion film or with a perm selective membrane of ion exchange character. In case of biocatalytic sensors a reaction layer is applied in front of the measuring surface resulting a thicker diffusion layer. The current signal obtained with conventional amperometric detection is determined by the rate of electroactive material transport. The thick diffusion layer of a chemically modified electrode can slow down the transport resulting small amperometric signal. In our experiments trying to solve different analytical tasks with biocatalytic electrodes we became interested to extend the measuring range of these sensors towards the lower concentrations [4]. It had been expected, that periodic interruption of the electrode reaction during detection would result in higher Faraday current. That is periodically interrupted amperometry with an appropriate program wave giving time for reloading the diffusion layer increasing the signal can lower the detection limit.

Adeloju et al. [13] used two potential step measuring program in their study with urea electrode prepared with conductive polymer entrapped urease enzyme. The program composed of measuring and resting steps. It provided improvement in sensitivity, in dynamic measuring range and in lower limit of detection. In a previous publication Senda and Yamamoto reported about measuring urea with an amperometric ammonia gas sensor using pulse technique [14]. Theoretical considerations helped to achieve improved sensor and measuring program.

In order to investigate the accessible detection limit extension or other possible advantages of this detection method, experiments were carried out in our laboratory with hydrophilic membrane coated platinum and carbon paste (CP) electrodes. In the pulsed amperometric measuring programs used in these experiments no electrode cleaning, electrode activation and conditioning pulses were included. There was only a measuring pulse with electrode potential needed for the detection and a resting potential section allowing time for the diffusion to reload the depleted layer at the electrode surface. The analytical properties of the pulsed detection were compared with those of the conventional DC amperometry. This short paper is about our results obtained with hydrogen peroxide and ascorbic acid detection.

2. Experimental

2.1. Chemicals

Analytical grade reagents were purchased from Sigma. In all experiments Sörensen buffer pH 7.33 (a phosphate buffer containing sodium-chloride) was used as back ground electrolyte. All the solutions were prepared with deionized water (specific conductivity $< 0.5\ \mu S\ cm^{-1}$). Ascorbic acid and hydrogen peroxide served as model compounds.

2.2. Instrumentation

One channel of a bipotentiostat (Elektroflex, Szeged, Hungary, type: EF437) was used for periodically interrupted and conventional amperometry. The voltage signal of the potentiostat, representing the current was digitalized by a PCLab812PG type (Advantech, Taiwan) AD/DA card. Home made software written in Microsoft Visual Basic 6.0 for CA and PIA measurements was used.

2.3. Electrodes

Disc shaped platinum and carbon paste electrodes with 2 and 4 mm diameter, respectively, made in our laboratory have been used. For making the electrodes platinum wire was purchased from Goodfellow (UK) and part of it was inserted into a hole of a Teflon tip of a rotating disc electrode (Radiometer Copenhagen, Denmark, Type CTV101). The electrode was used after proper polishing sequentially with 1, 0.3 and 0.05 μm Al_2O_3 polishing powder (Micropolish II, Buehler, US). Every time before use polishing was repeated, but only with 0.05 μm powder.

Standard procedure [15] was used for making in 1.9:1 ratio of highly purified graphite powder to Nujol and Union Carbide carbon powder (Type OD 1–2 μm Aldrich). A rotating electrode tip was used with a 4 mm diameter hole in the center as carbon paste electrode body.

In order to have a diffusion layer on the measuring surface – when it was needed – wet household cellophane dialysis membrane (pore size 12,000–14,000 Da; Visking) or porcine intestinal membrane was stretched over the surface and tied on with a thread.

AgCl coated silver wire inserted into the cell was the reference (quasi-reference electrode) and a large surface Pt was selected as counter electrode.

2.4. Periodically interrupted amperometric measuring program and method

The electrode potential–time program and the timing of the data collection used in the PIA measurements are showed in Fig. 1. As it can be seen, a square shaped wave is applied continuously with switching the potential of the working electrode between E_1 and E_2 with t_1 and t_2 timing. E_1 is the potential where the electrode process needed for detection proceeds while E_2 is the resting potential with no electrode reaction. There is a current measuring period t_4 indicated with a thicker section in the current curve. In order to exclude capacitive current, data collection started just after a certain time (t_3) of the potential change (typically 25 ms). The current intensity data collected for t_4 time (usually 10 ms selected after optimization study, see later) were summed up and averaged in order to reduce noise, and were plotted against the time.

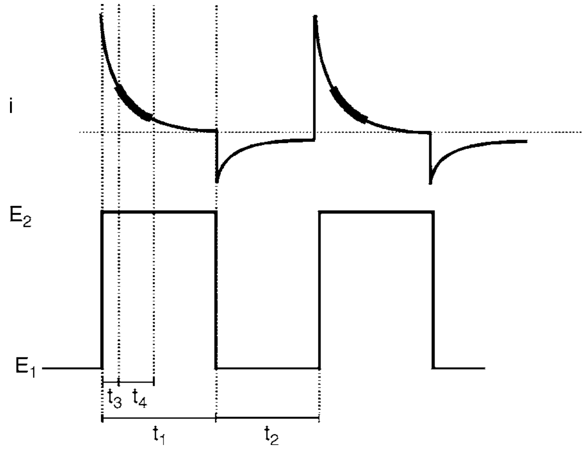

Fig. 1. Scheme of the measuring program used for PIA measurements. E_1, resting potential; E_2, measuring potential; t_1, measuring pulse length (200 ms); t_2, resting period (1500 ms); data collection starts at t_3 (25 ms), and its duration is t_4 (typically 10 ms).

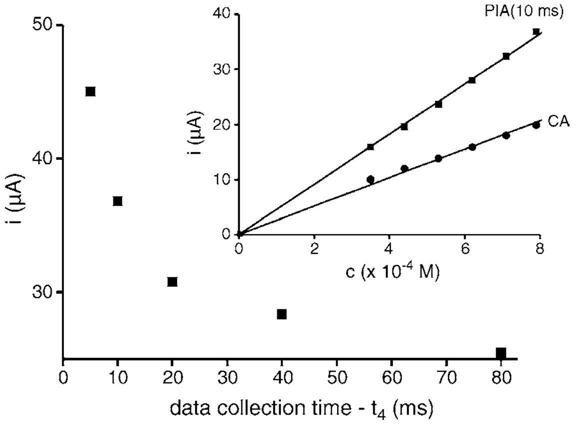

Fig. 2. Optimization of the data collection time length. Graph of the recorded current intensity at a H_2O_2 concentration of 7.93×10^{-4} M. 10 ms data collection time was selected for PIA experiments. Insert: calibration curves at different H_2O_2 concentrations taken with the PIA (10 ms long data collection time) and with CA method.

3. Results and discussion

3.1. Principles

The PAD or PCD methods usually use three or more controlled potential steps, responsible for different functions. In this special pulsed amperometry called PIA, beneficial for coated electrodes applies only one step measuring potential and a resting one. Its advantage can easily be seen considering the Cottrell experiment. That is chronoamperometric current–time curves taken in quiescent solution with a planar electrode usually show the Cottrell behavior. As the concentration of the electroactive species decreases in the vicinity of the electrode surface, that is as the diffusion layer gets more and more exhausted the current (i_t) decreases in time.

$$i_t = nFAc_0\sqrt{\frac{D}{\prod t}}$$

where n is the number of electrons taking part in the electrode process, c_0 the bulk concentration, D the diffusion coefficient, A the electrode surface area, and F is the Faraday charge. According to the equation the longer the time the smaller differences can be expected between i_t values obtained with solution of different concentration.

In conventional amperometry the electrode is continuously polarized. Therefore if a membrane coated electrode would be used its diffusion layer is expected to be partially exhausted. Giving time for the diffusion to reload the layer would result in higher current.

3.2. Optimization of PIA measuring method

It was necessary to optimize the length of data collection period within the measurements. To do this, calibrations were done in the concentration range of (0–7.93) × 10^{-4} M by adding H_2O_2 in the buffer solution. The data collection time was varied between 5 and 80 ms. The results obtained in 7.93×10^{-4} M solution are showed in Fig. 2 where the averaged current is plotted against the t_4 data collection time. As it can be seen the longer the t_4 the smaller the current measured. This means that data collected in a shorter period of time is advantageous to reach a lower limit of detection. However, under a shorter period only a less data could be collected and averaged thus the signal became noisier. As an optimal compromise 10 ms has been chosen for t_4 and used later on.

3.3. Applying PIA method on model compounds

In order to study the effect of PIA method. Rotating disc measurements were performed with dialysis membrane coated carbon paste electrode. In one case the electrode was placed in buffer solution (10 ml of Sörensen buffer). 400 mV versus Ag/AgCl reference measuring potential was set and the current was recorded using 1000 rpm electrode rotation rate. Certain time instant known volume of known standard ascorbic acid solution doses (25 μl of 100 mM) were added into the cell. After additions the current increased stepwise. The experiment was repeated with the only difference of changing the conventional amperometric detection to PIA one. The recordings obtained are compared in Fig. 3.

Current–ascorbic acid concentration are compared in Fig. 4. As it is clearly seen, in both cases linear calibration curve is obtained within the concentration range of (0–1.24) × 10^{-4} M. However, in case of PA the slope is about 10 times higher than in case of CA. These results prove that significant improvement of sensitivity can be obtained in measurements with membrane coated electrodes by replacing the very popular amperometric detection with a properly tailored PIA method.

In order to estimate the detection limit for ascorbic acid the calibration curves were drawn into the micromolar range and intersection of the curve (extrapolated part with the $c = 3 \times \sigma$ line (σ is the standard deviation, calculated from 50 measuring points) was accepted as lowest concentration accessible with the method also shown. Thus, the detection limit with

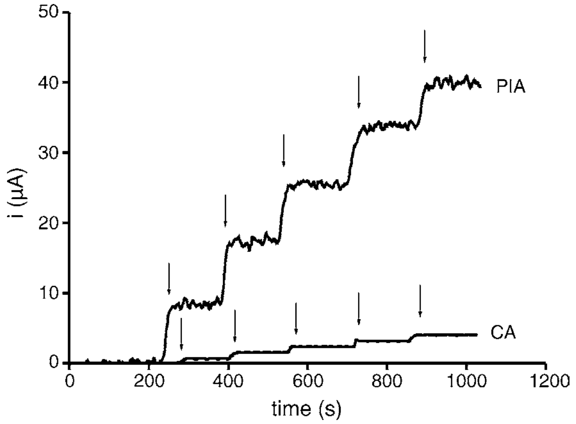

Fig. 3. Comparison of the recording obtained with of chronoamperometry and periodically interrupted amperometry. In both cases five times 25 µl of 100 mM ascorbic acid doses were added into 10 ml buffer. Resting electrode potential $E_1 = 0.0$ V, measuring electrode potential $E_2 = 400$ mV vs. Ag/AgCl reference. One thousand data points were used to get the averages and no filtration was used ($t_3 = 25$ ms, $t_4 = 10$ ms, resting period $t_2 = 1500$ ms) (electrode rotation rate 1000 rpm).

the PA method for ascorbic acid is given 4.5×10^{-7} M (Fig. 4 insert).

Similar experiments were made with hydrogen peroxide. It is worth mentioning here that H_2O_2 detection has a special importance since several oxido-reductase enzymes based – reaction layer coated – biosensors form analytic signal by amperometric oxidation of H_2O_2, product of the enzymatic reaction. The potential of the working electrodes was set to +600 mV versus Ag/AgCl reference electrode when CA method was used. For PIA measurements $E_1 = +600$ mV and $E_2 = 0$ mV versus Ag/AgCl reference electrode were set.

Calibration plot was taken in a H_2O_2 concentration range of 0–1.3 mM with CA and PIA methods. Serial addition of microliters of 0.88 M hydrogen peroxide was done into 10 ml Sörensen buffer. Fig. 5 shows the current–concentration plots. In this case one can also clearly see that the slope of the calibration in case of PIA is about two times higher then in case of CA. This proves that a remarkable improvement in the sensitivity can be achieved by the PIA technique.

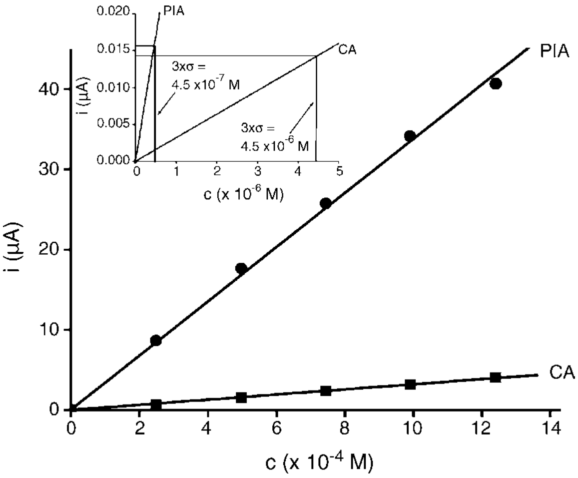

Fig. 4. Calibration curves with chronoamperometry and periodically interrupted amperometry for ascorbic acid using carbon paste electrode (1000 rpm). Insert: enlargement of the calibration curves in the micromolar range for determination of the 3σ based lower limit of detection.

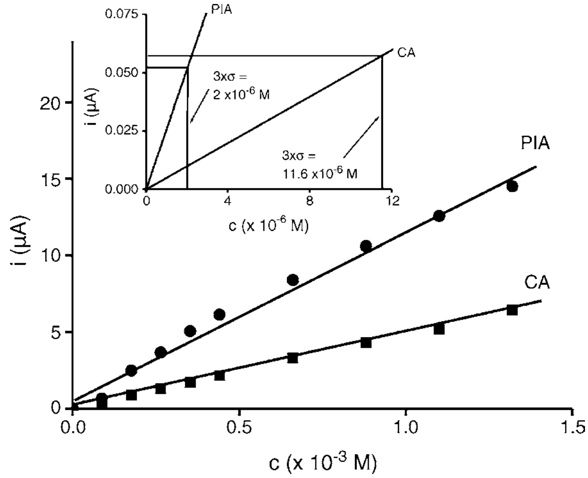

Fig. 5. H_2O_2 calibration curves taken with Pt electrode in case of CA and PIA methods. The resting potential was 0.0 mV and the measuring potential was +600 mV in PIA. The other parameters were the same as with ascorbic acid measurements. Insert: enlargement of the calibration curves for H_2O_2 in the micromolar range for estimation of 3σ based lower limit of determination.

The limit of detection for hydrogen peroxide was calculated with the same method as shown before (DL $\approx 3\sigma$). The insert in Fig. 5 shows the resulting graph. As a result in case of the PIA the value of 2.02 µM can be obtained, that is about 5.7 times lower as we got by using the chronoamperometric method.

4. Conclusions

A periodically interrupted amperometric (PIA) method was used to measure ascorbic acid and hydrogen peroxide with hydrophilic membrane coated carbon paste and platinum electrodes in convective media. The conventional amperometric and periodically interrupted amperometric measuring techniques were compared. The results showed the advantage of PIA in the cases investigated. The signal increased and the lower limit of detection decreased significantly when the CA method was changed to PIA detection in case of membrane coated electrodes. The results encourage us to investigate capabilities of PIA detection in studies aiming the lower detection limit of chemically modified electrodes.

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