Voltammetric and flow injection amperometric determination of insulin, uric acid, and ascorbic acid at the electrode modified by iridium oxides

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ABSTRACT

Aim: Mixed valence oxides of iridium, immobilized on surface of carbon electrodes by potentiodynamic electric sedimentation demonstrate electric catalytic activity and oxidation of insulin, uric acid (UA), and ascorbic acid. This leads to the reduction of overvoltage of considered compounds oxidation and increasing of their oxidation current relatively to current of modifier oxidation. Materials and Method: Possibility of jointed voltammetric determination of insulin, UA, and ascorbic acid on glassy carbon electrode modified by iridium oxides (IrO$_x$s) is established. Difference of potentials of these compounds oxidation peaks is 300 mV. Method of flow injection amperometric determination of insulin and UA on dual planar electrode modified by IrO$_x$s is proposed. Results: Linear dependence of analytic signal from concentration of organic compounds is observed in interval of $5 \times 10^{-8}$ to $5 \times 10^{-4}$ М for insulin and of $5 \times 10^{-7}$–$5 \times 10^{-3}$ М for UA. Developed method of flow injection amperometric determination on insulin and UA is distinguished by high sensitivity, repeatability, as well as rapidity, and productivity of analysis method (up to 480 determinations per hour). Conclusion: Proposed method was used for the determination of insulin and UA in human saliva.

KEY WORDS: Chemically modified electrode, Dual planar electrode, Electric oxidation of insulin, Flow injection analysis of human saliva, Iridium oxides, Uric acid and ascorbic acid

INTRODUCTION

Chemically modified electrodes (CMEs) with catalytic properties are frequently used as non-enzyme sensors at determination of biologically active substances my method of voltammetry. Chemical modification of electrode surface gives it specific properties promoting increase of sensitivity, selectivity, and repeatability of method. Special attention is paid to electrodes modified by electric catalytic method. Precious metals are frequently used as immobilized catalysts.

In clinical diagnostics, determination on insulin, uric acid (UA), and ascorbic acid (AA) is an important task because changes of their concentrations are characteristic for a range of diseases. Insulin is polypeptide hormone secreted by β-cells of Langherhans islets of pancreas. It maintains unchanging concentration of glucose in blood and is a direct participant of carbohydrates metabolism.$^{[1]}$ Determination of blood insulin is important for diagnostics of various types of sugar diabetes, hypoglycemic conditions, obesity, and selection of optimum dosage of medicines at the treatment of patients.

It is known that insulin resistance may reduce renal release of UA, which leads to increase of UA blood level and development of renal, liver, endocrine, and cardiovascular diseases. It its turn, besides participation in carbohydrate exchange, AA promotes insulin production and control of sugar level in diabetes patients. This is why control of AA blood level is also very important.

Thus, the development of fast, simple, and selective methods for the determination of insulin, UA, and AA in biologic fluids represents an undoubted interest. For quantitative determination of insulin, UA, and AA, various physical-chemical methods are used, such as titrimetry,$^{[2,3]}$ spectrophotometry,$^{[4-6]}$ chemiluminescence,$^{[7,8]}$ potentiometry,$^{[9,10]}$ high-efficiency liquid chromatography (HELC),$^{[11-13]}$ as well as enzyme immunoassay.$^{[14]}$ Along with advantages, these methods have some limitations. Low sensitivity and selectivity are drawbacks of

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spectrophotometry, potentiometry, and titrimetry. Immunological methods allow to determine with high precision only one component in course of analysis, and in conditions of HELC are determining several components but after preliminary preparation of samples, frequently including preliminary derivatization. Besides, these methods are complicated in conduction, time, and cost taking. This is namely why voltammetric method with CMEs that have the advantage of simplicity in experiment conduction, high analytical characteristics, and non-expensive equipment represents interest.

In this work we studied catalytic activity of iridium oxides (IrO$_x$), electrically sedimented on surface of glass-carbon electrode (GC) or on surface of operational electrodes of dual planar electrode (DPE) alla electric oxidation of insulin, UA and AA in stationary mode and possibility of use of modified DPE for sensitive and selective ampermetric determination of these compounds at joint presence in conditions of flow-injection analysis (FIA).

**METHODS**

At conduction of researches in stationary conductions was used bipotentiostat DropSens μSTAT400 (Spain) with three-electrode cell, including operational electrode of GC or CME. Chlorine-silver electrode performing function of comparative electrode and platinum wire used as auxiliary electrode.

Modification of operational carbon electrodes surface by oxides IrO$_x$ was conducted by method of potentiodynamic electrolysis. Electric sedimentation was conducted from solution containing iridium chloride (IrCl$_3$) on the background of 0.1 M H$_2$C$_2$O$_4$.

In course of recording of cyclic voltammetograms (CVA), rate of potential overlapping (v) equal to 20 mV/s was used.

Flow injection system included peristaltic pump PERIMAX 12 (Germany), injector, flow electrochemical cell, and bipotentiostat DropSens μSTAT400. Diagram of flow injection system is shown in Figure 1. Analyte was detected in electric chemical flow cell of “wall-jet” type with PE with one and two operational electrodes (DPE) manufactured by company DropSens (Spain) or modified PE and DPE.

Phosphate buffer solution with pH level of 6.9 served as background electrolyte.

**RESULTS AND DISCUSSION**

**Insulin**

In non-modified GC, on the background of phosphate buffer solution with pH 6.9, insulin oxidizes with high overvoltage (at $E > 0.80$ V); at this poor repeatability of voltammetric characteristics is observed [Figure 2a, curves 2, 3, 4]. At electric oxidation of insulin, in its molecule occurs oxidation of phenol groups of thyrosine fragment by scheme:[17]

**UA**

Process of UA oxidation on carbon electrodes in water media is irreversible. Peal of UA oxidizing is registered at potential of 0.65 V. Mechanism of UA oxidizing is complicated.[18,19] It is suggested that at pH 7.0 occurs oxidizing of UA to unstable UA diimine that transfers into 4,5-diol UA with further degradation to allantoin.[18,19] Electric oxidation of UA in neutral medium is usually presented by the following scheme:[18,19]

**Ascorbic Acid**

Electrochemical oxidation of AA occurs with loss of 2 protons and 2 electrons and with formation of dehydro AA$_2$.[20]
At oxidation of AA at GC on anode branch of CVA, poorly repeatable irreversible peak extended along the axis of potentials is observed. Potential of peak maximum is 0.70 V in neutral medium.

At this, dependencies of current value from concentration of considered organic compounds are linear in the following concentration intervals: Of $1 \times 10^{-3}$–$5 \times 10^{-4}$ M for AA and UA and of $1 \times 10^{-4}$–$1 \times 10^{-5}$ M for insulin, which is much higher than reference values in biological fluids.

At scanning of potential of 0.0–1.0 V on GC with electrically sedimented (IrO$_x$-GC), two current maximums are registered [Figure 2b, curve 1]. At potential of 0.50 V occurs iridium oxidation from Ir (II) to Ir (III), and at E > 0.80 V forms IrO$_x$ (IV).

At anode branch of CVA, at oxidation of organic compounds on electrode IrO$_x$-GC, one peak which height increases with increasing of analyte concentration is observed. As example, in Figure 2b, voltammetryogram of insulin oxidation on electrode IrO$_x$-GC is illustrated. Potential of insulin oxidation peak maximum $E$ 0.70 V corresponds to potential of modifier. At this, reduction of overvoltage of insulin oxidation is 120 mV. Oxidation of AA and UA on this CME occurs at $E$ 0.1 and 0.4 V, respectively (with reduction of overvoltage for 250 and 600 mV). To determine the nature of electrochemical process, measuring of current of peaks obtained at different rates of potential overlapping was conducted. On the basis of value of angle coefficient $\tan \beta = \Delta E/\Delta \log v$ (Semerano coefficient) was established that electrochemical process was complicated by chemical reaction ($\tan \beta = 0.35$). Obtained data allow to make a conclusion that studied process is catalytic.

Due to need of analytical control of insulin, UA and AA in biological objects, possibility of voltammetric definition of considered compounds at joint presence on CME was considered. It was established that on anode voltammetryogram obtained for three-component system of AA, UA, and insulin on electrode IrO$_x$-GC, three clearly expressed peaks are observed [Figure 3]. Difference of potentials of oxidizing peaks for AA and UA, UA, and insulin is 300 mV. Thus, with electrode IrO$_x$-GC, selective definition of these compounds at joined presence is possible.

High repeatability of analytic signal registered at electrode IrO$_x$-GC is established. Calculated values $S_i$ for current of oxidation of considered compounds do not exceed 5.0% (at $n = 20$ and $C = 5 \times 10^{-3}$ M for AA and UA and $5 \times 10^{-4}$ M for insulin).

Obtained results were used at analysis of saliva in flow injection system. AA is contained in saliva in insignificant amounts (in nanomolecular concentrations), so the possibility of simultaneous flow injection amperometric definition of two-component system insulin/UA by DPE modified by IrO$_x$-DPE was considered.

Electrochemical and hydrodynamic conditions of analytical signal registration on PE with electrically sedimented IrO$_x$-PE in flow injection system were preliminary optimized for UA and insulin. To do this, speed of liquid flow ($u$) and value of overlapped potential at potentiostatic mode of FIA signal registration. The following operational conditions for registration of considered compounds FIA signal were selected: $u = 4.2$ ml/min and $E = 0.40$ and 0.70 V for UA and insulin, respectively. Range of linear dependence of current value on analytes concentration and respective equations of regression are shown in Table 1.

At multiple repeating of voltammetryograms, a fine repeatability of respond was obtained. Calculated values $S_i$ for CME response do not exceed 2.0% (at $n = 10$, $P = 0.95$ and $C = 5 \times 10^{-3}$ M for UA and $5 \times 10^{-4}$ M for insulin).
Table 1: Analytical characteristics for definition of organic compounds on carbon electrodes modified by IrO₅ on the background of phosphate buffer with pH 6.9

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Electrode</th>
<th>Range of concentrations, mol.L⁻¹</th>
<th>Equation of regression lgC=a+b×10⁻⁴</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>n</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>lgC; (i, mea; C, mol.L⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>

By voltammetric method with CME

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Electrode</th>
<th>Range of concentrations, mol.L⁻¹</th>
<th>Equation of regression lgC=a+b×10⁻⁴</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IrO₅-CG</td>
<td>5×10⁻⁵ + 5×10⁻⁴</td>
<td>2.8±0.1</td>
<td>0.999</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td></td>
<td>5×10⁻⁷ + 5×10⁻⁴</td>
<td>3.3±0.1</td>
<td>0.999</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td></td>
<td>5×10⁻⁴ + 5×10⁻³</td>
<td>3.3±0.1</td>
<td>0.998</td>
</tr>
</tbody>
</table>

By amperometric method with CME in conditions of FIA

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Electrode</th>
<th>Range of concentrations, mol.L⁻¹</th>
<th>Equation of regression lgC=a+b×10⁻⁴</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IrO₅-PE</td>
<td>5×10⁻³ + 5×10⁻⁴</td>
<td>2.3±0.2</td>
<td>0.998</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UA</td>
<td></td>
<td>5×10⁻¹ + 5×10⁻¹</td>
<td>1.36±0.05</td>
<td>0.995</td>
</tr>
</tbody>
</table>

CME: Chemically modified electrodes, FIA: Flow injection analysis

Table 2: Results of flow injection amperometric definition of insulin and UA on double PE with particles of iridium in saliva n=3, P=0.95, tᵣ=4.3

<table>
<thead>
<tr>
<th>Analyte</th>
<th>C, M (for healthy man)</th>
<th>Found, M</th>
<th>Sᵣ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>(0.3–1.7)×10⁻⁴</td>
<td>(1.4±0.9)×10⁻⁷</td>
<td>0.06</td>
</tr>
<tr>
<td>UA</td>
<td>(0.3–1.7)×10⁻⁴</td>
<td>(0.64±0.03)×10⁻⁴</td>
<td>0.05</td>
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UA: Uric acid, PE: Planar electrode

CONCLUSIONS

Implementation of DPE modified by IrO₅ as amperometric detectors makes possible conduction of multicomponent analysis in FIA conditions, while the use of miniature flow electrochemical cell promoted reduction of samples, reagents consumption, and forming waste.

ACKNOWLEDGMENTS

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REFERENCES


SUMMARY

The use of catalytic response of electrode from D PE modified by IrO₅ for flow injection amperometric definition of insulin and UA promotes increase of sensitivity, selectivity, repeatability, as well as increase of rapidity, and productivity of analysis.

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