A voltammetric sensor for simultaneous determination of tryptophan and 5-hydroxytryptophan

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Abstract. The present work proposes a method for simultaneous determination of tryptophan and 5-hydroxytryptophan on the carbon-bearing electrode modified with multiwall carbon nanotubes and polyfolic acid. Analytical signals are conditioned by oxidation of tryptophan and 5–hydroxytryptophan from the complex containing polyfolic acid. Preparation of the electrode consists in the formation of the B9 vitamin film on the surface of the carbon-bearing electrode, modified with multiwall carbon nanotubes by the method of cyclic voltammetry. The detection limit of tryptophan and 5-hydroxytryptophan is $1 \times 10^{-7}$ M.

1. Introduction
Tryptophan (Trp) is one of the essential amino acids playing a key role in biological metabolism and protein secretion in living organisms. It acts as a precursor for the synthesis of such neurotransmitters in the human body as niacin, melatonin and serotonin. A part of tryptophan is used for protein secretion in the human body and the other part – for 5–hydroxytryptophan metabolism. Trp deficit leads to such mental disorders as anxiety, depression, insomnia, etc. Otherwise, the excess of Trp in the body can cause nausea, lack of appetite. Trp cannot be synthesized in the body; therefore, to maintain a sufficient quantity of neurotransmitters, there must be Trp obtained from foodstuff, medicaments and biologically active dietary supplements (BADS) [1–3].

At present, to determine tryptophan and 5–hydroxytryptophan, different methods allowing evaluating their content in a wide range of concentrations are used: capillary electrophoresis [4], fluorescence spectroscopy [5], chromatographic methods [6–8], mass spectrometry [8], NMR [9]. However, from the analytical point of view, the application of the mentioned methods requires time-consuming sample preparation, expensive equipment and reagents, thorough cleaning of the latter, as well as highly qualified staff. In this connection experts pay much attention to electrochemical methods due to their simplicity, high selectivity, sensitivity and low cost of equipment.

When using voltammetric methods, the detection limit of tryptophan and 5-hydroxytryptophan on inert electrodes is quite high; therefore, to increase sensitivity, electrodes are modified. Frequently, metal nanoparticles [10–14], carbon nanotubes and nanofibers [14–16], cyclodextrins [17], graphene and graphene oxide [18–19], amino acids and the vitamin B complex [20–21] are used for this purpose.

In [10] a glassy-carbon electrode modified with nanoparticles of gold and carbon nanotubes was used. This electrode has a good capacity for electrochemical oxidation of tryptophan at the expense of adsorption. The linear range of the determined concentrations is from $5.0 \times 10^{-6}$ M to $100.0 \times 10^{-6}$ M
with the detection limit of $3.0 \times 10^{-6}$ M. The authors of [11] presented the method of detection of tryptophan using the electrode, in the production of which the main components were ionic liquid and multiwall carbon nanotubes. The electrode surface was activated by potential cycling. During tryptophan oxidation the electrode was not destructed. The range of the determined concentrations was $5.0 \times 10^{-6} - 1.0 \times 10^{-3}$ M. A highly selective electrochemical sensor, representing a hybrid structure of aluminium oxide, graphene and copper, which was produced by electrodeposition of copper nanoparticles on aluminium oxide nanofibers, encapsulated into graphene, was presented in [12]. This electrode was used for simultaneous determination of adrenaline, acetaminophen and tryptophan with a low detection limit of 0.027, 0.012, 0.009 µM, respectively. The detection method of tryptophan on the glassy-carbon electrode modified with silver was described in [13]. In the optimal conditions of the experiment, oxidation peak current is linearly dependent on the Trp concentration in the range from $1.0 \times 10^{-7}$ to $1.0 \times 10^{-4}$ M with a detection limit of $4.0 \times 10^{-8}$ M.

The glassy-carbon electrode modification with multiwall carbon nanotubes having a nanopalladium coating to detect 5-hydroxytryptophan was described in [14]. The linear range of concentrations for this electrode was $2 - 400 \times 10^{-6}$ M. The method of direct simultaneous detection of L-tryptophan, L-tyrosine and L-cysteine with modified electrodes, made of carbon mixture, and carbon nanofibers was given in [15]. The linear ranges of detection of tryptophan, tyrosine and cysteine were $0.1 \times 10^{-6} - 119 \times 10^{-6}$, $0.2 \times 10^{-6} - 107 \times 10^{-6}$ and $0.15 \times 10^{-6} - 64 \times 10^{-6}$ M, respectively. The electrode modified with multiwall carbon nanotubes was presented in [16]. The detection limit was $2.7 \times 10^{-8}$ M. The modified electrode in [17] was formed by applying a dripping tryptophan coating on the film made of chitosan on the surface of the glassy-carbon electrode, modified with multiwall carbon nanotubes (detection limit was $1.0 \times 10^{-9}$ M). The authors of [18] developed an enantioselective voltammetric sensor system based on glassy carbon electrodes modified with composites of polyarylenephtalide with α-, β- and γ-cyclodextrins for selective recognition and determination of tryptophan enantiomers. The method of direct simultaneous detection of L-tryptophan (Try) using red wood eucalypt as an environmentally friendly reducing agent to synthesize nanoparticles of the reduced oxide of graphene and gold was presented in [19]. The synthesized nanocomposite was used as a sensitive and electroactive substrate on the surface of the print-screen electrode. The linear range of concentrations was $0.5 - 500 \times 10^{-6}$ M with a detection limit of $4.0 \times 10^{-6}$ M. The preparation of the print-screen electrode modified with hydroxyapatite and graphene oxide to determine L-tryptophan with a detection limit of $5.5 \times 10^{-6}$ M was demonstrated in [20].

All the mentioned methods have their advantages, but they are not free from shortcomings associated with expensive materials and a time consuming process of the formation of the active layer surface. The modification of the electrode surface with films of amino acids and the vitamin B complex can be an available and simple method of determination [21–22]. In [21] the L-lysine film was applied on the glassy-carbon electrode by potential cycling in the range of 0.0 – 1.9 V in the phosphate buffer solution (pH was 8.6) in the presence of copper ions. The linear range of concentrations for this electrode was $0.69 - 50 \times 10^{-6}$ M. In [22] the B12 vitamin film was used to determine methionine due to bonding of methionine and the B12 vitamin, and methionine was reduced to homocysteine during anodic potential sweep. The detection limit of methionine was $1 \times 10^{-7}$ M.

Since the vitamin B complex has good electrochemical characteristics, we propose using them when detecting tryptophan and 5-hydroxytryptophan. Folic acid (vitamin B9) participates in the folate cycle in the human body. The folate cycle is a complex multicomponent combination of reactions owing to which a number of vital processes proceed in the body. Folates play a key role as their reduced form participates in the transfer of single-carbon fragments («formyl» –CHO; «methenyl» –CH=; «methylene» –CH2; «methyl» –CH3) from serine, tryptophan and histidine to nucleic acids (DNA, RNA). These fragments are essential elements of synthesis of purine and pyrimidine bases, which determines the significance of tetrahydrofolic acid during cell fission as the development of this process seems to be impossible without the synthesis of nucleic acids.

Vitamin B9 is capable of electropolymerization at low potentials. In [23] a study on finding optimal conditions for formation of the stable film of polyfolic acid was undertaken. For this, the graphene-
chitosan composite was applied on the surface of the electrode, filmed with polyfolic acid, to prevent its wash-out from the electrode surface. In this work we propose using folic acid as a modifier for the carbon-bearing electrode.

2. Experimental

2.1. Apparatus
All electrochemical measurements were made using the voltammetric analyser TA-LAB (NPO “Tomanalit”). Voltammetric studies in the direct current mode were conducted in the three-electrode cell. The modified carbon-bearing electrode was used as an indicating electrode; chloride-silver electrodes in 1 M of KCl were used as auxiliary and reference electrodes. The nanotube suspension was prepared in the ultrasound bath Sapfir – 2.8 TTTS.

2.2. Reagents
In this work folic acid (Sigma), tryptophan, 5-hydroxytryptophan (Sigma, USA) were used. Multiwall carbon nanotubes (MWCNT) were purchased from the company Aldrich. Buffer solutions were prepared using standard-titres (pH 4.01; 6.86; 3.56; 9.18). All the chemical agents were prepared using deionized water, obtained on Sartorius of the arium®pro grade. All the experiments were conducted at room temperature.

2.3. Preparation of modified electrodes
The suspension of multiwall carbon nanotubes was prepared in the following way: the MWCNT sample was oxidized in the mixture of 1:1 HNO₃ (conc) and H₂SO₄(conc) up to complete evaporation of acids. Then the oxidized nanotubes were rinsed with deionized water and dissolved in dymethylformamide. The suspension aliquot (5 ml) was applied on the electrode surface up to full evaporation of the solvent. The folic acid film was applied on the surface of the carbon-bearing electrode by cyclic potential sweep in the range of −1.4 V ∆ 0.5 V at 80 mV/s during 9 cycles from 0.02 M of the alkaline solution of the B₉ vitamin.

3. Results and discussion

Figure 1. a) Folic acid polymerization on the carbon-bearing electrode modified with MCNT. b) Current-voltage curves of tryptophan and 5–hydroxytryptophan against the background of Na₂HPO₄ – NaH₂PO₄ pH = 6.86 (1 – 5–hydroxytryptophan and tryptophan with the concentration of 1.0 ×10⁻⁸ M; 2 – 5–hydroxytryptophan and tryptophan with the concentration of 2×10⁻⁸ M).
Figure 1a shows the process of folic acid polymerization during cyclic potential sweep. The peak current at a potential of $-0.75$ V increases, which indicates the growth of the film on the carbon-bearing electrode modified with MWCNT. Under the action of electric current, amine being in the pteridine ring of the folic acid molecule is charged up to a positive cation-radical. This cation-radical joins the free amine of the pteridine group. As a result of the deposition of polyfolic acid, the electrically active surface of the electrode increases (according to the Randles–Sevcik equation, it was 0.038 cm$^2$).

To determine a possible mechanism of the electrode process and optimal parameters of registration of the analytical signal, the influence of pH and the composition of the background solution on the potential and peak current, parameters of concentration of amino acids and registration of analytical signals was studied. Peak signals for tryptophan and 5-hydroxytryptophan are revealed on the phosphate-buffered saline (pH 6.86) presented in figure 1b. With such pH value, tryptophan and 5-hydroxytryptophan, according to the conducted calculations (figure 2), are in the form of a zwitter ion.

![Figure 2](image-url)  
**Figure 2.** Mole fractions of tryptophan and 5-hydroxytryptophan by the acid group: I $- \alpha$(COOH), II $- \alpha$(COO$^-$) and the basic group: III $- \alpha$(NH$_3^+$), IV $- \alpha$(NH$_2$).

![Figure 3](image-url)  
**Figure 3.** The mechanism of concentration and oxidation of tryptophan on the electrode modified with polyfolic acid.
To evaluate the possible mechanism of the electrode process, the influence of pH of the solution on the potentials of anodic peaks of tryptophan and 5–hydroxytryptophan was analyzed. The slope of 44.9 mV/pH for tryptophan and 41.5 mV/pH for 5-hydroxytryptophan points to an equal number of protons and electrons participating in the electrochemical reaction. Linearized dependences of peak current on the square root of the potential sweep rate have the following form: $y = 0.467x - 0.1536$; $y = 0.0506x + 0.0988$ for tryptophan and 5–hydroxytryptophan, respectively. Analysis of these dependencies using the Randles–Sevcik equation allows drawing a conclusion about participation of two protons and two electrons in the electrode reaction. The possible mechanism of concentration and electrodissolution by the example of tryptophan can be presented as the following scheme (figure 3).

The optimal conditions of obtainment of analytical signals of tryptophan and 5–hydroxytryptophan are $E_{el}=-0.1$ V, $W=170$ mV/s, $t_{el}=120$ s. The electrodes can be cleaned electrochemically at a potential of +1.0 V. The electrode functions steadily during 50 cycles, after which the surface requires renewal.

In the optimal conditions the dependencies of the value of anodic peaks of tryptophan and 5–hydroxytryptophan can be described by the following equations: $I = 0.088C + 5.992$ ($R^2 = 0.996$) and $I= 0.098C + 3.715$ ($R^2 = 0.998$), respectively, in the range of $0.84\times10^{-7} - 38.00\times10^{-6}$ M. The minimal determined concentration is $1\times10^{-7}$ M.

The validation of this method was evaluated through the technique “introduced-obtained” and by calculation of the rate of opening ($R$), the results of which are given in the table. Values of $R$ are close to 100%, which indicates the absence of a significant systematic error.

| № of sample | Content in a sample [mg/kg] | Introduced [mg/kg] | Obtained [mg/kg] | $\Delta$ [mg/kg] | $R$ [%] |
|-------------|-----------------------------|-------------------|-----------------|-----------------|--------|
| 1           | 1.00                        | 1.0               | 1.98±0.73       | 0.98            | 98     |
| 2           | 1.30                        | 1.0               | 2.35±0.49       | 1.05            | 105    |
| 3           | 1.22                        | 1.0               | 2.30±0.85       | 1.08            | 108    |

4. Conclusion
This work presented the method of simultaneous determination of tryptophan and 5–hydroxytryptophan on the carbon-bearing electrode modified with MWCNT and polyfolic acid. A possible mechanism of the formation of the analytical signal was proposed. High sensitivity, simplicity of the electrode modification make the proposed method promising for practical determination of amino acids in biologically active dietary supplements and medicaments. The detection limit of tryptophan and 5-hydroxytryptophan was $1\times10^{-7}$ M.

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