Using of Carbon Nanotubes for Fabrication of Printed Electrodes and Their Employing in DNA Analysis

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In the beginning of the 21st century the complete structure of human genome was recognized. Since then the importance of the rapid testing of DNA nucleotide sequences is increasing. Except specialized genetic tests, rapid detection of biological weapons, environmental monitoring and forensic medicine the quick DNA analysis is approaching to common use. In contemporary numerous scientific institutions are searching for low cost, rapid and easy-to-use tools for such purposes. Electrochemistry belongs to promising methods in DNA analysis. The electrochemical behaviour of nucleic acids has been studied for more than 40 years. The first publication describing the nucleic acids determination by using the mercury electrode was published in 1960 [1, 2]. The measurement on mercury electrodes allow the determination of small amount both unmodified and electrochemically labeled nucleic acids. Nucleic acids gave two signals: i) redox signal of adenine and cytosine, and ii) oxidative signal of guanine. Moreover, electroactivity of all four nucleic bases was observed by using of carbon electrodes [3-5]. The aim of this work was to find and optimize the electrochemical methods for DNA detection on nanocarbon screen printed electrodes.

Chemicals

DNA and standards of nucleic acid bases was obtained from Reanal. The other chemicals were purchased from Sigma-Aldrich, unless stated otherwise. Materials for electrodes fabrication: the carrier substrate was from ceramic (Alumina-96% Al_2O_3), pastes 5545, 9635-HG, 4460 (ESL) and 5874, 7105, BQ221 (DuPont). Multiwalled carbon nanotubes (Sigma Aldrich) mixed with organic binder and homogenized to thixotropic paste.

Screen-printed electrodes fabrication

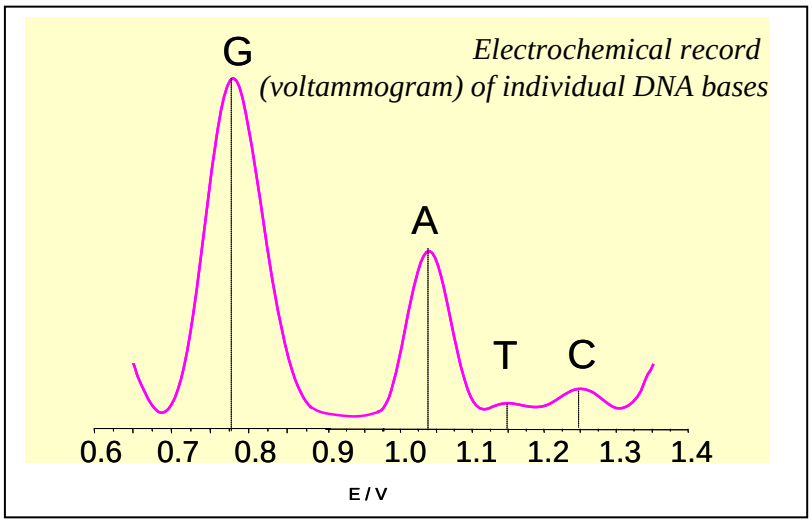
For fabrication of printed electrodes the semi-automatic screen-printing machine UL-1505A Tesla, laser AUREL AUTOMATION ALS 300L, hot-flow furnace HS 62A and inline furnace BTU were utilized. The used materials were: working electrode – carbon, reference electrode – Ag/AgCl, auxiliary electrode – Pt, isolation – dielectric paste, leads and pads – AgPd. The area of the working electrode was 0.5 mm^2.

Electrochemical measurements

The electrochemical measurement was carried out with multi-mode potentiostat BioStat (ESA, Inc. USA). It is four-channel system with three operating modes per channel (amps, volts, and temp). The system is connected through data bus USB to personal computer. To the first channel of the potentiostat the home made apparatus was connected. This apparatus consists of basic plate on which the connector TX721 1115 with pins spacing 2.54 and the connector 0039532035 from the manufacturer Molex with pins spacing 1.25 mm are placed. The connectors are designed for connection of two different screen-printed electrodes. For data processing the software BioStat and MS Excel was used. The parameters of the analysis were

as follows: channel number 1, sampling frequency 20 sps (samples/s), filter order 5. The working electrode potential was set according to previous analyses at carbon paste electrode. Phosphate buffer pH 6.0 was used as supporting electrolyte. All experiments were carried out at room temperature.

Measurement itself was performed according to the following scheme. For base lane establishing 10 µl of the supporting electrolyte was introduced on the electrodes surface, then 2 µl of the sample was added and the changes of current were determined. The electrode surface was mechanically polished with redistilled water and filter paper. Primarily we optimized experimental conditions for determination of nucleic acid bases by using of carbon paste electrodes. Under the optimal conditions (0.1 M phosphate buffer, pH = 6.0, frequency of 50 Hz and time of accumulation 120 s) we analyzed all four nucleic acid bases – adenine, guanine, cytosine and thymine using nanocarbon screen printed electrodes and well repeatable data were obtained (Fig. 1). Potentials of certain bases were as follows: cytosine C ~ 1.3 V; thymine T ~ 1.2 V; adenine A ~ 1.0 V; guanine G ~ 0.7 V. The detection limits evaluated at the fabricated electrodes were 0.2 µM for cytosine, 1.5 µM for guanine, 0.8 µM for thymine and 1.5 µM for adenine. The optimized procedure was further used for DNA analysis, where the signals of single bases were distinguished. When the denatured and native DNA was measured, it was observed, that the signals of temperature denatured DNA was of about 40 % higher in comparison to native DNA. The detection limit of native DNA was of about 1.5 µg/ml and the detection limit of denatured DNA was 0.5 µg/ml. The surface of the working electrode was enhanced by using of the carbon nanotubes created in plasma with nickel catalyst. The using of nanotubes increased the electrochemical response of 40 % and the detection limit was markedly lowered.



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