Available online at www.sciencedirect.com

ScienceDirect

Procedia Chemistry 1 (2009) 1011–1014

Procedia Chemistry

www.elsevier.com/locate/procedia

Proceedings of the Eurosensors XXIII conference

Modification of Working Electrode Surface with Carbon Nanotubes as an Electrochemical Sensor for Estimation of Melting Points of DNA

Jaromir Hubalek[a,*], Jan Prasek[a], Dalibor Huska[b], Martin Adamek[a], Ondrej Jasek[c], Vojtech Adam[b,d], Libuse Trnkova[e,f], Ales Horna[g], Rene Kizek[b]

[a]*Department of Microelectronics, Faculty of Electrical Engineering and Communication, Brno University of Technology, Udolni 53, CZ-602 00 Brno, Czech Republic*

[b]*Department of Chemistry and Biochemistry, and* [d]*Department of Animal Nutrition and Forage Production, Faculty of Agronomy, Mendel University of Agriculture and Forestry, Zemedelska 1, CZ-613 00 Brno, Czech Republic*

[c]*Department of Physical Electronics,* [e]*Department of Chemistry, and* [f]*Research Centre for Environmental Chemistry and Ecotoxicology, Faculty of Science, Masaryk University, Kotlarska 2, CZ-611 37 Brno, Czech Republic*

[g]*Tomas Bata University, T.G. Masaryka 275, CZ-762 72 Zlin, Czech Republic*

Abstract

Screen-printed with three electrode system was used in this study. A working electrode has been printed from carbon nanotubes based paste on silver layer modified with nano-patterned structures for the first case. In the second case, vertically aligned carbon nanotubes were grown on the Au working electrode. The process of the nanotubes growing was tested to create homogenous and high density carbon nanotubes layer directly on the thick-film silver layer. Based on the characterization of electrodes, we used Au based for detection of nucleic acids. Moreover, we were able to estimated melting points of DNA.

Keywords: Carbon pase electrodes; Voltammetry; DNA; Melting points

1. Introduction

Carbon nanotubes (CNTs) belong to the most promising nano-materials, because they show unique electronic, mechanical and chemical properties [1] that lead to many applications. They can be prepared by arc discharge [2], laser ablation [3] and chemical vapor deposition [4] methods. For industrial applications it is desirable to produce vertically aligned CNT films with uniform properties [5,6]. Most of techniques used for industrial fabrication work at low pressure requiring vacuum systems. However for industrial application it would be desirable to work at atmospheric pressure. In electrochemical application the CNTs are usually used as material for polymer or epoxy based composites [7]. CNTs can be also used as a material for working electrodes, because carbon based electrodes

* Corresponding author. Tel.: +4-205-414-6193; fax: +4-205-414-6298.
E-mail address: hubalek@feec.vutbr.cz.

doi:10.1016/j.proche.2009.07.252

are attractive for electrochemical measurements today due to tend to be miniaturize. Among various types of miniaturized electrodes, screen printed electrodes have several advantages including low cost of preparation, easy-to-use and mechanical stability. Screen printing technique belongs to the thick-film technology (TFT) was utilized for production of hybrid integrated circuits, later was used for fabrication of special integrated circuits, small series of nonstandard integrated circuits and prototypes [8]. The advantage of TFT sensors are low dimensions, good reproducibility, mechanical and electrical properties of electrodes and well accessible and ecological fabrication process [8]. The technology uses cermet or polymer pastes, firing or drying at furnace. The process is simple and undemanding for equipment and environment (clean room is not necessary) resulting to low fabrication costs.

Screen-printed carbon based electrodes are low-cost option in environmental, analytical and bioanalytical chemistry [9]. The aim of this work is to prepare the sensors with carbon nanotubes mixed in the paste and compared with improved CNT grown directly on sensor's electrode using synthesis of the vertically aligned CNTs in atmospheric microwave plasma torch discharge as was published in [5,6] and use in field of electrochemical analysis. Particularly, we used the electrodes for estimation of DNA melting points.

2. Material and Methods

2.1. Sensor design and fabrication

The thick-film screen-printed electrodes were designed and published as a sensor on alumina for electrode material properties measurement [10]. An Ag based paste has been designed for leads and connector. The reference electrode material has been designed also based on Ag that can be electrochemically covered by AgCl layer after the main sensor fabrication process. Auxiliary electrode material is suggested from a Pt paste but the material of each electrode can be easily changed by a use of other type of the paste. Central circular working electrode was modified by screen printed CNT layer or by atmospheric microwave plasma torch discharge.

The TFT material used for auxiliary electrode, reference electrode and conductive layer was ESL 9912-D paste and for dielectric layer ESL 4913-G paste. For the working electrode the ESL 4913-G paste (Ag) and ESL 8844-G paste (Au) were used (all pastes were ESL ElectroScience, UK).

2.2. Working electrode modification with multi-wall carbon nanotubes

The working electrode with multi-wall CNTs is created in several steps (Fig. 1A). Commercially available multi-wall carbon nanotubes (MWCNTs) were mixed with epoxy-based vehicle to homogenize the paste with thixoptrophic properties. The prepared paste was printed over the Ag working electrode. Then the printed layer was dried at 150°C. The bottom layer of the working electrode is formed from the Pt or Au Ag cermet paste to prevent intermetallic reaction with catalyst during the carbon nanotubes fabrication. The carbon nanotubes grow vertically aligned on the working electrode surface forming nanopatterned structure. This electrode modification by the nanotubes is homogeneous and the electrode surface is completely covered with high density amount of the nanotubes. To deposit the carbon nanotubes on the working electrode its surface was modified by thin nickel or ferric layer (10-20 nm) created by magnetron sputtering or by thermal vacuum evaporation served as a catalyst in the CNTs growth and argon, hydrogen and methane were used as working gases. The gas flow rates were controlled by electronic flow controllers. Microwave power of 400 W (2.45 GHz, 2 kW max. power) is supplied by a microwave generator and transmitted by a waveguide through a coaxial line to a hollow nozzle electrode. A ferrite circulator protects the generator against the reflected power by rerouting it to the water load. The coaxial line and the electrode accommodated a dual gas flow. Argon (1500 sccm) flew through a central opening (1 mm) and the deposition mixture, H2/CH4 (42/430 sccm), was added by a set of holes in the outer housing. The plasma expands from the central nozzle forming a torch discharge. A quartz tube, 40mm in outer diameter, separates the discharge from surrounding atmosphere. At the bottom it is sealed by a Teflon piece to the flange of the outer coaxial conductor. At the top it is closed by an upper flange with an exhaust tube and a sealed feed through for a substrate holder. The substrate holder is another quartz tube, 18 millimeters outer diameter, fixed at the upper flange. This tube is closed at its top by a quartz window. At the opposite side, i.e. close to the discharge nozzle, two slits are cut through the tube. The sensor was facing the torch during the deposition. Its temperature was measured by Raytek

Thermalert TX pyrometer from the back side. After the plasma torch was ignited by an auxiliary rod electrode in flowing argon, the sensor was placed at the desired deposition distance (30-50 mm) from the nozzle and the deposition mixture H_2/CH_4 was added. The deposition temperature (T_d) was regulated by the deposition distance and it was varied from 950 to 1100 K. The deposition time (t_d) was 1 to 15 minutes.

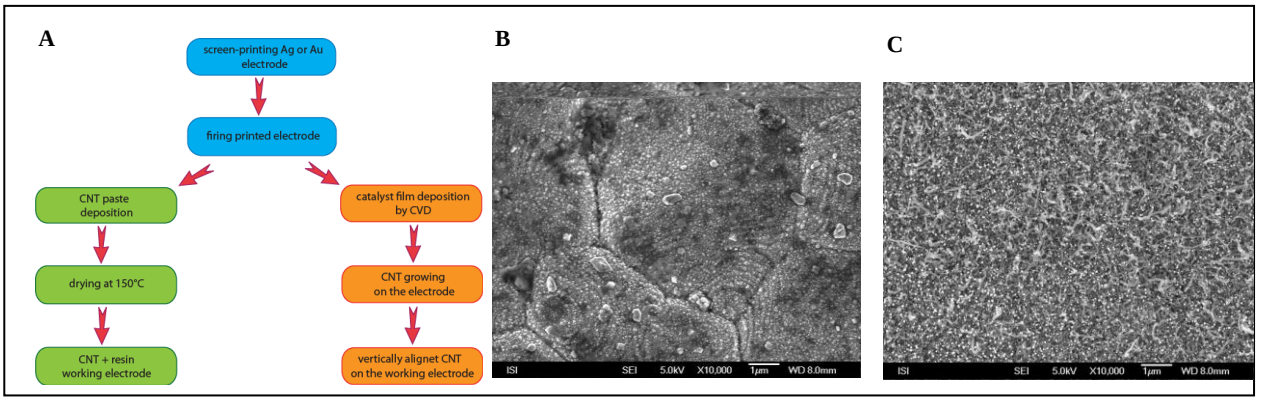

Figure 1: (A) Fabrication process of working electrode preparation. SEM micrographs of the working electrode with Au based thick-film paste (B) covered by Ni catalyst, (C) with the carbon nanotubes deposit.

2.3. Scanning electron microscope

Surface morphology of the deposits was studied by a scanning electron microscopy (SEM) with JEOL 6700F microscope equipped with an EDX analyzer. The acceleration voltage was usually 5 kV and working distance was in the range of 8-9 mm. The pictures were taken at several points from the surface of the working electrode.

2.4. Chemicals

All chemicals used were purchased from Sigma Aldrich (Sigma-Aldrich, USA) unless noted otherwise. DNA was extracted from pullet erythrocyte dissolved in acetate buffer (500 μg/ml).

2.5. Electrochemical measurements

Electrochemical measurements were performed with AUTOLAB Analyzer (EcoChemie, Netherlands) connected to VA-Stand 663 (Metrohm, Switzerland), using a standard cell with three electrodes. A screen-printed electrodes modified with MWCNTs was employed as the working electrode. An Ag/AgCl/3M KCl electrode served as the reference electrode. Glassy carbon electrode was used as the auxiliary electrode. For smoothing and baseline correction the software GPES 4.9 supplied by EcoChemie was employed. To 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. All electrochemical measurements were carried out in the presence 0.2 M Britton-Robinson buffer (pH = 4.5) at room temperature.

3. Results and Discussion

We prepared two sensor sets with different paste materials. The first set was based on the Ag paste and the second on the Au paste. The surface is relatively homogenous without porosity and lumps. The catalyst layer (10 nm of Ni or Fe) was evaporated or sputtered on the top of the sensor working electrode in vacuum. This issue was confirmed by scanning electron microscope analysis of the carbon nanotubes deposits (Fig. 1B,C). Based on the characterization of the electrodes, we used Au based for detection of nucleic acids. Cyclic voltammograms (500 μg/ml dsDNA) measured at scan rates 250, 500 a 750 mV/s are shown in Fig. 3A. Oxidation signal of guanine (G_{ox})

was detected at 0.6 V and oxidation signal of adenine (A_{ox}) at 0.9 V. The data was baseline corrected to obtain well developed voltammetric signals. It is a common knowledge that nucleic acids well interact with a carbon electrode and the signals measured are proportional to concentration of the target molecules. Dependences of peak heights on accumulation time are shown in Fig. 3B. The height of the peaks changed markedly. These changes can be associated with charge of the electrode surface and charge of polynucleotide chain. Moreover, we used the electrode to estimate melting point for polynucleotide chain. We attempted to distinguish ssDNA and dsDNA. This can be done by easy-to-use test with heat denaturation of dsDNA. The changes in heights of voltammetric DNA signal during heat treatment are shown in Fig. 3C. From the dependence melting point as 83 °C was estimated. This was in good agreement with spectroscopic value measured.

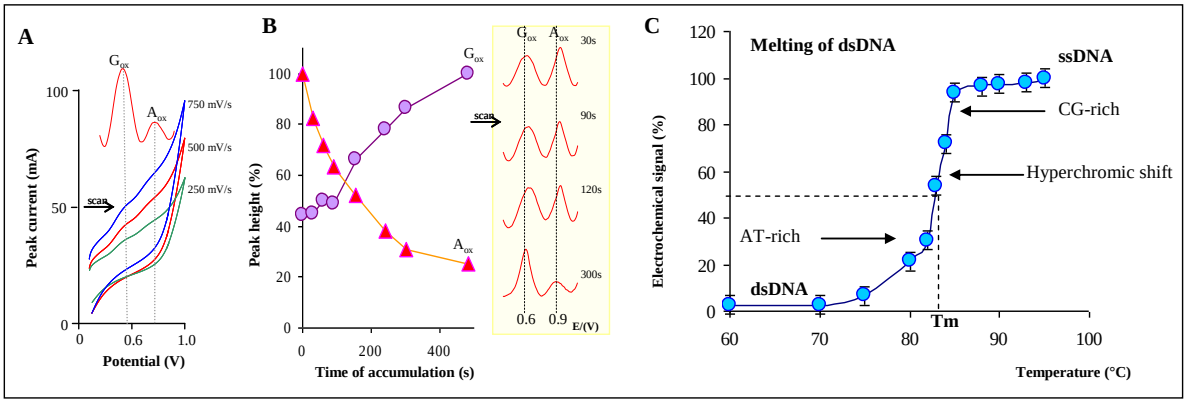

Figure 3: Results of electrochemical detection of DNA on the screen-printed electrode.

Acknowledgements

Financial support from the grants GAAV 1QS201710508, GACR 102/09/P640 and GACR 102/08/1546 is highly acknowledged.

References

1. P.M. Ajayan; Chem. Rev., 99 (1999), pp 1787-1799.
2. S. Iijima; Nature, 354 (1991), pp 56-58.
3. A. Thess, R. Lee, P. Nikolaev, H.J. Dai, P. Petit, J. Robert, C.H. Xu, Y.H. Lee, S.G. Kim, A.G. Rinzler, D.T. Colbert, G.E. Scuseria, D. Tomanek, J.E. Fischer, R.E. Smalley; Science, 273 (1996), pp 483-487.
4. C.J. Lee, D.W. Kim, T.J. Lee, Y.C. Choi, Y.S. Park, Y.H. Lee, W.B. Choi, N.S. Lee, G.S. Park, J.M. Kim; Chem. Phys. Lett., 312 (1999), pp 461-468.
5. L. Zajickova, P. Synek, O. Jasek, M. Elias, B. David, J. Bursik, N. Pizurova, R. Hanzlikova, L. Lazar; Applied Surface Science, 255 (2009), pp 5421-5424.
6. L. Zajickova, M. Elias, O. Jasek, V. Kudrle, Z. Frgala, J. Matejkova, J. Bursik, M. Kadlecikova; Plasma Phys. Control. Fusion, 47 (2005), pp B655-B666.
7. M. Pumera, A. Merkoci, S. Alegret; Sens. Actuator B-Chem., 113 (2006), pp 617-622.
8. J.P. Hart, S.A. Wring; Trac-Trends Anal. Chem., 16 (1997), pp 89-103.
9. O.D. Renedo, M.A. Alonso-Lomillo, M.J.A. Martinez; Talanta, 73 (2007), pp 202-219.
10. M. Lanz, D. Schurch, G. Calzaferri; J. Photochem. Photobiol. A-Chem., 120 (1999), pp 105-117.
11. P. Babula, D. Huska, P. Hanustiak, J. Baloun, S. Krizkova, V. Adam, J. Hubalek, L. Havel, M. Zemlicka, A. Horna, M. Beklova, R. Kizek; Sensors, 6 (2006), pp 1466-1482.
12. J. Prasek, M. Adamek, J. Hubalek, V. Adam, L. Trnkova, R. Kizek; Sensors, 6 (2006), pp 1498-1512