SUPPORTING INFORMATION

Sensitive Sepiolite-Carbon Nanotubes Based Disposable Electrodes for Direct Detection of DNA and Anticancer Drug –DNA Interactions

Arzum Erdem[1,*], Filiz Kuralay[1,2], H.Evren Çubukçu[3], Gulsah Congur[1], Hakan Karadeniz[1], Ece Canavar[1]

[1] Analytical Chemistry Department, Faculty of Pharmacy, Ege University, 35100, Bornova, Izmir
[2] Department of Chemistry, Ordu University, 52200, Ordu
[3] Department of Geological Engineering, Hacettepe University, 06800, Beytepe, Ankara
TURKEY

1. Experimental

1.1 Apparatus

All experimental measurements were carried by using AUTOLAB – PGSTAT 302 electrochemical analysis system supplied with a FRA 2.0 module for impedance measurements and GPES 4.9 software package (Eco Chemie, The Netherlands). For electrochemical measurements, differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) were used. The three electrode system was consisted of the pencil graphite electrode (PGE), an Ag/AgCl/KCl reference electrode (BAS, Model RE-5B, W. Lafayette, USA) and a platinum wire as the auxiliary electrode. The EIS measurements were performed in the faraday cage (Eco Chemie, The Netherlands).

1.2 Chemicals

The stock solutions of calf thymus DNA (ctdsDNA) (1000 mg/L) were prepared with Tris-EDTA buffer solution (10 mM Tris-HCl, 1mM EDTA, TE, pH 8.00) and kept frozen. More diluted solutions of DNA were prepared with 0.50 M acetate buffer containing 20 mM NaCl (ABS; pH 4.80).

The raw rock material used was a natural brown sepiolite from Türktaciri deposit (Sivrihisar, Turkey).

Carboxylic acid (80 – 90%) functionalized single-walled carbon nanotubes (diameter, 4 – 5 nm; length 500 – 1500 nm bundles) were purchased from Aldrich.

Mitomycin C (MC) [8] was purchased from Sigma (shown in Figure S1). The stock solution of MC was prepared in ultra pure distilled water. More diluted solutions of MC were prepared with 0.02 M Tris-HCl buffer solution containing 20 mM NaCl (pH 7.00, TBS).

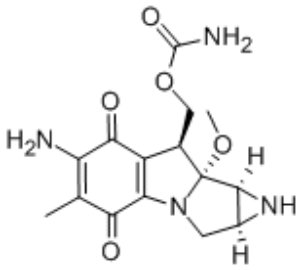

Figure S1. The chemical structure of MC.

Other chemicals were in analytical reagent grade, supplied from Sigma and Merck. All stock solutions were prepared using deionized and autoclaved water.

1.2.1 Sepiolite (SEP) sample preparation

The raw rock material used was a natural brown sepiolite from Türktaciri deposit (Sivrihisar, Turkey). In order to obtain liberated sepiolite crystals for the experiments, the following sample preparation has been adopted: The rock sample has been left to dry in room temperature for 10 days to become suitable for crushing process. Dried rock fragments have been crushed by a jaw crusher and sieved to obtain a 5 mm size fraction, which was used for further processing. An aqueous suspension of sepiolite of 10% by weight with distilled water has been ground in a ceramic bill with 4000 rpm for 20 minutes. The ground suspension then sieved on 0.063 mm screen and dessicated at 110^{o}C for 24 hours. Following the dessication, a final powdering in agate mortar and sieving (0.063mm) have been applied to eliminate the lumps formed during drying. The size fraction used in the experiments is <0.063 mm.

1.2.2 SEM Characterization

Samples have been mounted on carbon tabs and coated with Au to provide a conducting film of ~7 nm. Carl-Zeiss EVO 50 EP Scanning Electron Microscope (Germany) equipped with an Everhart-Thornley type secondary electron detector has been used for imaging. The operating conditions were 20-30 kV accelerating voltage, 10-15 pA beam current with 4-5 mm working distance.

1.3 Procedure

All the experiments were done at room temperature. Unprepared PGEs, SEP modified PGEs and SEP-SWCNTs modified PGEs were used in each electrochemical detection cycle.

1.3.1 Preparation of SEP/SEP-SWCNTs Solution and SEP/SEP-SWCNTs Modified PGEs

Sepiolite solution was prepared by dissolving 2.0 mg/mL SEP in ultrapure and deionized water. The suspension was sonicated during an hour at room temperature and then unpretreated PGE was modified with sepiolite solution by passive adsorption for 1 h. These SEP modified PGEs were left drying by upside down for 5 min. For SWCNTs modification, 3 mg/mL SWCNTs were dissolved in DMF by sonication for 1 h. The SEP modified PGEs were immersed in SWCNTs solution for 1 hour. These modified electrodes were left to dry for 5 min.

1.3.2 DPV Measurements

1.3.2.1 DNA Immobilization onto the Surface of SEP and SEP-SWCNTs Modified PGEs and Electrochemical Detection of DNA:

Each modified PGEs were immersed into the vials containing 110 µL of 50 µg/mL of DNA solution in ABS for an hour. Each of the electrodes was then rinsed with ABS for 3 s before voltammetric transduction.

Voltammetric transduction:

DPV measurements were used for monitoring the guanine and adenine oxidation on unmodified PGE, SEP modified PGE, SWCNTs modified PGE and SEP-CNT modified PGE surface. DPVs were performed in ABS with three electrode system by scanning from +0.40 V to +1.20 V at the pulse amplitude; 50 mV and the scan rate; 50 mV/s.

1.3.2.2 Immobilization of MC onto PGEs and SEP-CNT Modified PGEs and Electrochemical Detection of MC:

SEP-SWCNTs modified PGEs were immersed into the vials containing different concentrations of MC prepared in TBS for 5 minutes according to passive adsorption process. After then, the electrodes were rinsed with TBS for 10 seconds. Voltammetric transduction was performed in ABS in order to monitor the MC oxidation signal.

1.3.2.3 Interaction of MC with DNA immobilized SEP-SWCNTs PGE and Electrochemical Detection of MC-DNA Interaction:

DNA immobilized electrodes were immersed into the vials containing 150 µg/mL MC prepared in TBS for interaction according to passive adsorption process. After 5 minutes, each electrode

was rinsed with TBS for 10 seconds. After the interaction process at the electrode surface, the voltammetric transduction was then performed in ABS to measure the oxidation signals of MC and guanine in the same conditions.

1.3.3 EIS Measurements

The surfaces of unmodified, SEP, CNT and SEP-CNT modified PGEs were characterized by EIS technique accordingly to the procedure given at below.

EIS measurements were performed in the presence of 2.5 mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ (1:1) mixture as a redox probe prepared in 0.1 M KCl. The impedance was measured in the frequency range from 10^5 Hz to 10^{-1} Hz in a potential of open-circuit value of +0.23 V, versus Ag/AgCl with a sinusoidal signal of 10 mV. The frequency interval divided into 98 logarithmically equidistant measure points. The respective semicircle diameter corresponds to the charge-transfer resistance, Rct, the values of which are calculated using the fitting programme AUTOLAB 302 (FRA, version 4.9 Eco Chemie, The Netherlands).

Results and Discussion:

The interaction of MC with dsDNA on SEP-SWCNTs modified PGE was investigated before/after the interaction process (Fig. 6). The voltammograms presented the oxidation signals of MC, guanine (G) and adenine (A) recorded about at + 0.83, + 0.99 and + 1.24 V respectively. After MC-DNA interaction for 7.5 min, a gradual decrease of 70.48 % , 91.80 % and 21.45 % was obtained correspondingly on MC, guanine and adenine signals (shown in Figure 6- a to a', b to b' and c to c' and Figure S2-A to B).

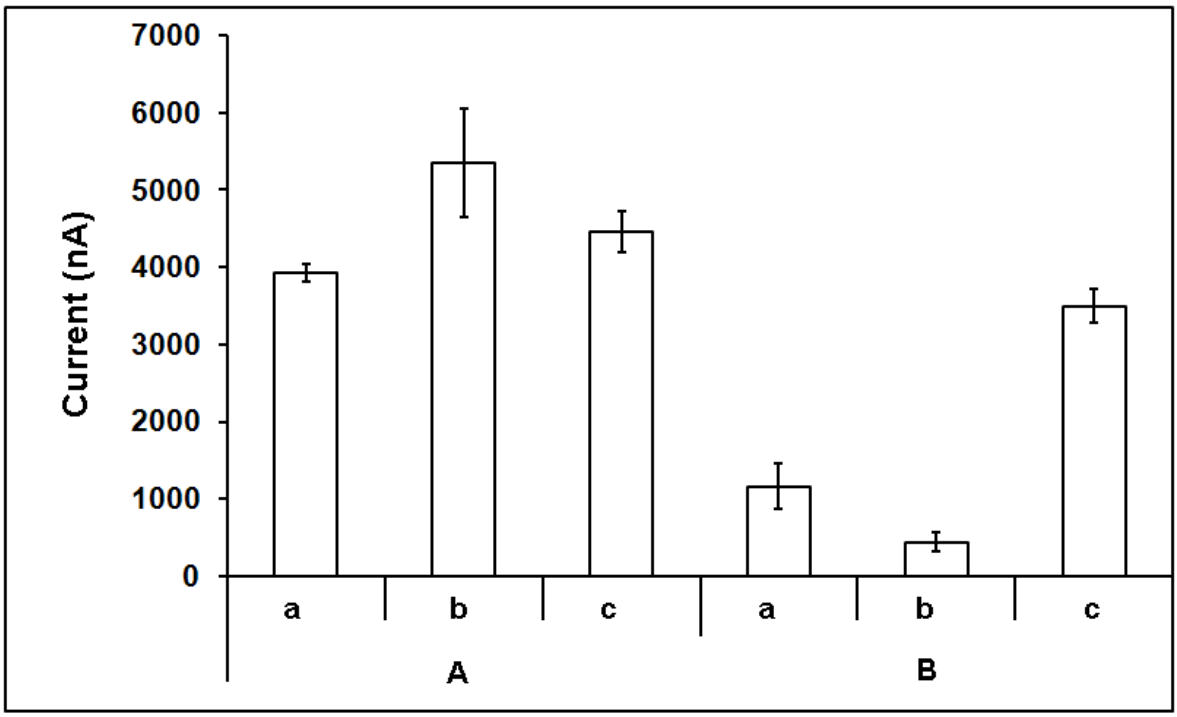

Figure S2. Histograms representing the oxidation signals of MC and DNA bases observed in the absence **(A)** , or presence **(B)** interaction between 150 µg/mL MC and 125 µg/mL ctDNA: **(a)** MC, **(b)** guanine, **(c)** adenine oxidation signals recorded by SEP-CNT modified PGE.