Electronic Supplementary Material (ESI) for Analytical Methods.

An electrochemical biosensor based on graphene oxide modified pencil graphite electrode for direct detection and discrimination of double-stranded DNA sequences

M. Ahmadi [a], F. Ahour [a*]

[a] Nanotechnology Research Center, Faculty of science, Urmia University, Urmia, Iran.

Corresponding author:

* F. Ahour

E-mail: f.ahour@urmia.ac.ir; Fax: +98 44-32752746

1. Preparation of GO

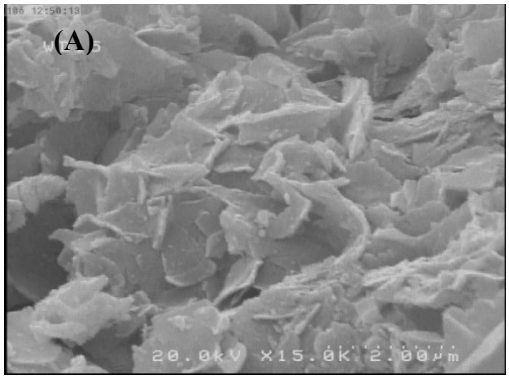
GO was prepared by the oxidation of graphite powder according to Hummers method with a modification of removing $NaNO_3$ from the reaction formula.[1] For the preparation of GO, graphite powder (3.0 g) was added to concentrated H_2SO_4 (70 mL) under stirring in an ice bath. Under vigorous stirring, $KMnO_4$ (9.0 g) was added slowly to keep the temperature of the suspension lower than 20 °C. Then, the reaction temperature was raised to 40 °C and vigorously stirred for about 0.5 h. After that, H_2O (150 mL) was added, and the solution was stirred for 15 min at 95 °C. Additional H_2O (500 mL) was added and followed by a slow addition of 15 mL H_2O_2 (30%), turning the color of the solution from dark brown to yellow. The reaction mixture was filtered and washed with 1:10 HCl aqueous solution (250 mL) to remove metal ions. The resulting solid was dried and diluted to 600 mL, making a graphite oxide aqueous dispersion. Finally, it was filtered off and the resultant graphite oxide aqueous dispersion was then diluted to 1.2 L, stirred overnight and sonicated for 30 min to exfoliate it to GO. The GO dispersion was then centrifuged at 3000 rpm for 40 min to remove the unexfoliated graphite.

Table 1S. The sequence of oligonucleotide used in this study

Name	Sequence
PNA probe (PHCV)	*ATG TAC CCC ATG AGG TCG GC*
DNA probe ($PHCV_{uni}$)	*5`- ATGTACCCCATGAGGTCGGC-3`*
HCV_{uni}	*5`-GCC GAC CTC ATG GGG TAC AT -3`*
Com-ds-DNA	*ATG TAC CCC ATG AGG TCG GC* | | | | | | | | | | | | | | | | | | | | | | *TAC ATG GGG TAC TCC AGC CG*
NC-ds-DNA	*TAA TGA GGG CTG CGG GTG GG* | | | | | | | | | | | | | | | | | | | | | | *CCC ACC CGC AGC CCT CAT TA*

2. Characterization of synthesized GO

Fig. 1S. (A) The SEM image of GO; (B) TGA image of graphite and synthesized GO



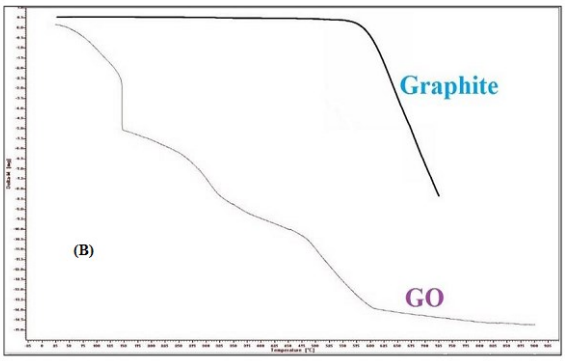
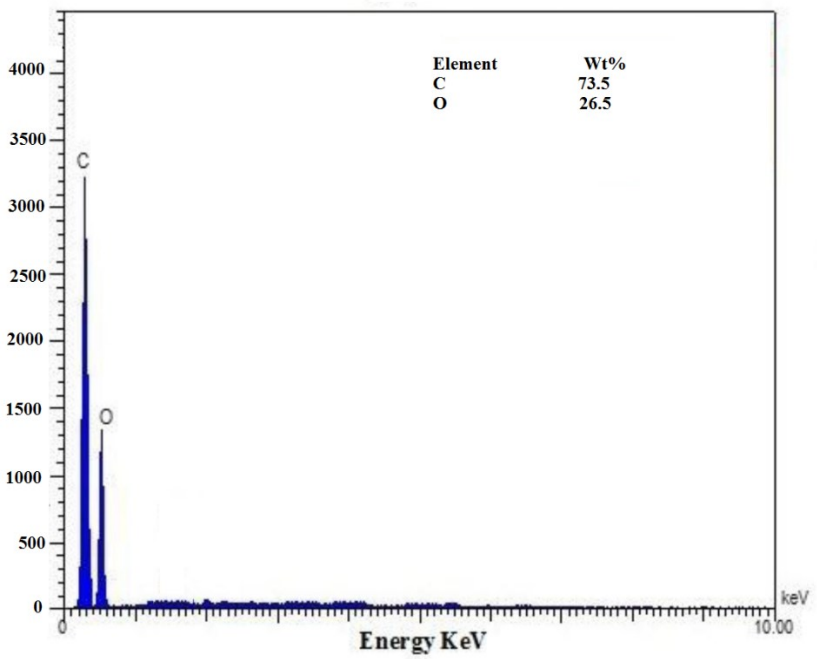
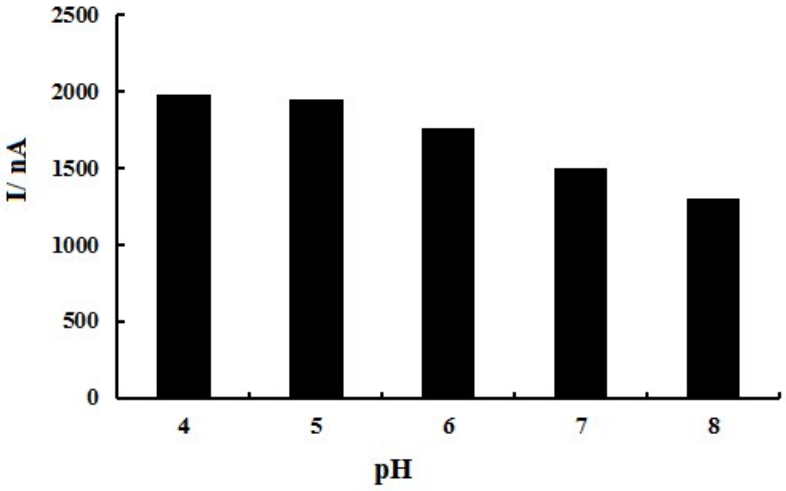


Fig. 2S. EDS analysis result of synthesized GO



3. Optmization of probe immobilization condition

Fig. 3S. Effect of PNA probe solution pH on the DPV signal of prepared electrode



4. Selectivity of the biosensor

Selectivity of the biosensor also examined in the mixed solution of complementary and non-complementary plasmid samples and results showed that PNA-GO/PGE could detect complementary plasmid in the present of non-complementary samples. This prove sequence specific and selective response of biosensor to ds-DNA.

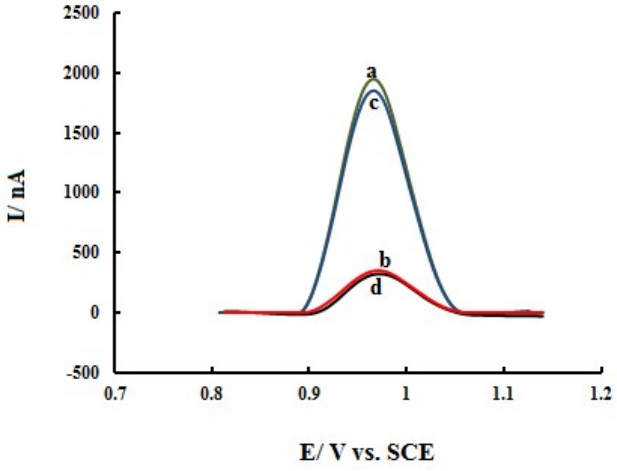


Fig. 4S. DPV of probe modified GO-PGE: **a** before, and after interaction with **b** ds-com plasmide, **c** with ds-NC plasmid, and **d** a mixture of complementary and non-complementary plasmid samples

References

1. J. Chen, B. Yao, C. Li, G. Shi, An improved Hummers method for eco-friendly synthesis of graphene oxide. Carbon 64 (2013) 225–229.