



ELECTROANALYTICAL STUDIES OF SYNTHETIC OLIGONUCLEOTIDES HYBRIDIZATION

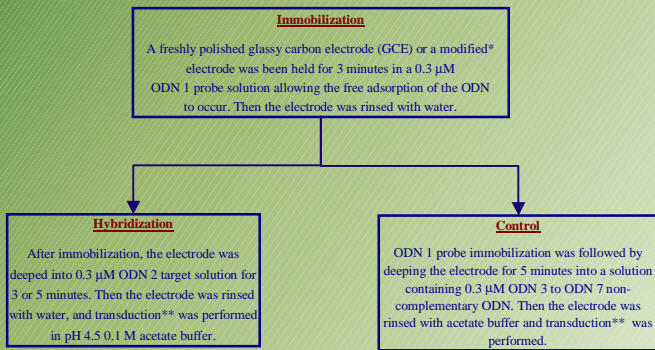
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The need for the analysis of gene sequences and monitoring microorganisms in medical, environmental or food control, the determination of the oxidative damage to DNA and the understanding of DNA interactions with molecules or ions led to the development of DNA-based biosensors. The DNA sequences are unique to each organism, so therefore any self-replicating biological organism can be discriminated using DNA hybridization. However, synthetic oligonucleotides (ODNs) are progressively replacing genomic and cloned DNA and are ideal chemical recognition elements, because the hybridization is highly sequence-selective. The present study deals with the characterization of complementary oligonucleotides (ODN) hybridization using electrochemical methods.

Probe	ODN 1	5' - GTAGATCACT - 3'
Target - complementary sequence:	ODN 2	5' - AGTGATCTAC - 3'
Control - non-complementary sequence:	ODN 3	5' - AAAAAA - 3'
	ODN 4	5' - AAAGAAAAAG - 3'
	ODN 5	5' - AAAAGGAGAG - 3'
	ODN 6	5' - GGGGCCCGG - 3'
	ODN 7	5' - CTTTTTCTTT - 3'



* **Modification** of the GCE surface has been achieved by applying a potential of +1.4 V for 2 minutes in a solution containing 0.3 μM ODN 1.

** **Transduction** was always carried out into pH 4.5 0.1 M acetate buffer using differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) at +0.5 V.

VOLTAMMETRY OF ADSORBED ODN 1

Three different procedures of GCE surface conditioning were estimated in order to optimize the ODN 1 immobilization conditions:

- 1st - various DPV at a freshly polished GCE were recorded in pH 4.5 0.1 M acetate buffer until a stable base line was obtained;
- 2nd - a potential of +1.4 V was applied during 2 minutes in supporting electrolyte to a freshly polished GCE;
- 3rd - a freshly polished glassy carbon surface was used.

The three DPV were compared. Very reproducible results and higher oxidation peaks of guanosine and adenosine were obtained without any previous conditioning of a freshly polished electrode surface.

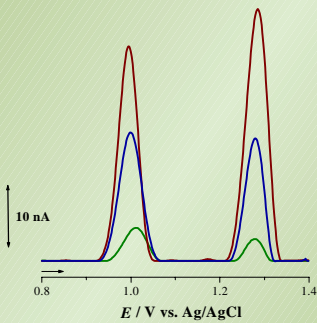


Figure 1 - Base line corrected DPV in pH 4.5 0.1 M acetate buffer after immobilization of ODN 1 at a GCE surface previously conditioned using the (—) 1st, (---) 2nd and (· · ·) 3rd procedure.

The effect of ODN 1 concentration and immobilization period on the guanosine and adenosine D.P.V peak currents was undertaken. Different probe concentrations ranging between 0.1 and 1.2 μM were assessed in connection with 10 seconds to 10 minutes of free adsorption time.

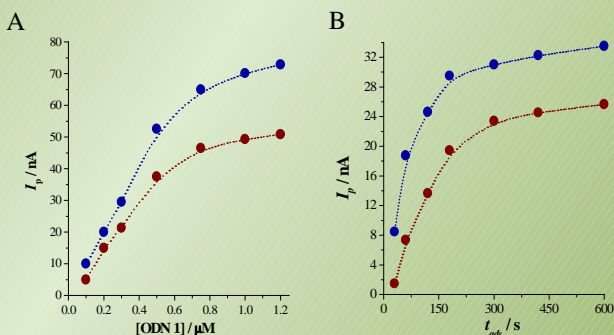


Figure 2 - Variation of (●) guanosine and (●) adenosine DPV oxidation peak currents, obtained in pH 4.5 0.1 M acetate buffer after free adsorption from a ODN 1 solution with: A) concentration for 3 min adsorption time, and B) adsorption time for a 0.3 μM ODN 1 solution.

Target molecules present in solution can hybridize and can also adsorb at the probe-uncovered electrode regions leading to an increase of the overall guanosine and adenosine concentration on the electrode surface. The non-specific adsorption strongly affects the results.

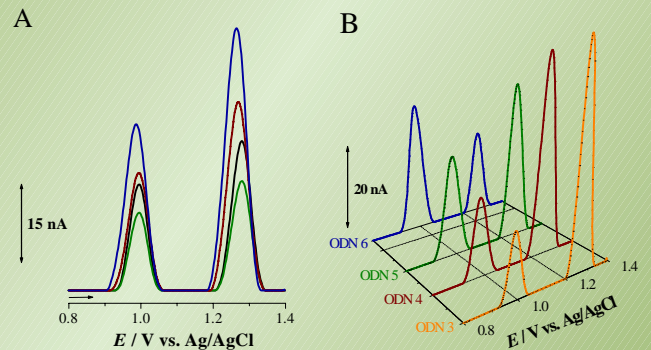


Figure 3 - Base line corrected DPV in pH 4.5 0.1 M acetate buffer obtained with a GCE after: A) (—) free adsorption for 3 min in 0.3 μM ODN 1, (---) incubation for 5 min of the ODN 1 immobilized at the GCE surface with the complementary target ODN 2, control experiments for 5 min in complementary (—) ODN 5 or (---) ODN 7, B) control experiments in 0.3 μM non-complementary solutions of (—) ODN 3, (---) ODN 4, (· · ·) ODN 5 and (· · ·) ODN 6. Scan rate 5 mV s⁻¹, pulse amplitude 50 mV, pulse width 70 ms.

VOLTAMMETRIC STUDIES OF HYBRIDIZATION ON AN ODN-MODIFIED GCE SURFACE

In order to reduce the contribution from non-specific adsorbed ODN sequences during hybridization, experiments using an ODN modification of the GCE surface were undertaken. After hybridization a decrease in the guanosine and adenosine oxidation currents occurs, as expected. The non-specific adsorption is considerably reduced when using an ODN-modified GCE.

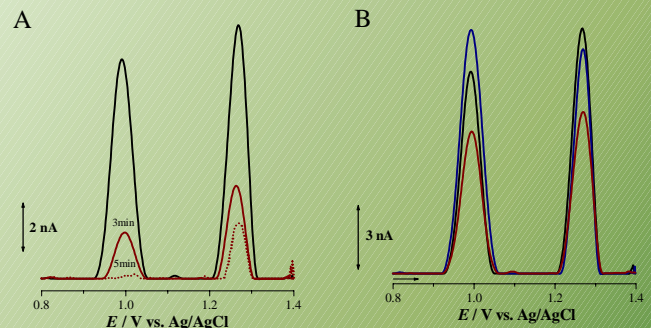


Figure 4 - Base line corrected DPV in pH 4.5 0.1 M acetate buffer obtained with an ODN-modified GCE after: A) immobilization of (—) ODN 1 and hybridization for (---) 3 min and (· · ·) 5 min in 0.3 μM ODN 2 and B) control experiments for 5 min in 0.3 μM: (—) ODN 5, (---) ODN 6 and (· · ·) ODN 7. Scan rate 5 mV s⁻¹, pulse amplitude 50 mV, pulse width 70 ms.

EIS CHARACTERIZATION OF HYBRIDIZATION AT AN ODN-MODIFIED GCE SURFACE

An almost pure capacitive behaviour was observed after immobilization of ODN 1. After hybridization an increase in the impedance was detected, corresponding to a decrease of the interfacial capacitance.

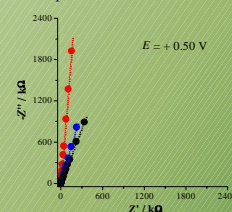


Figure 5 - Complex plane impedance spectra in pH 4.5 0.1 M acetate buffer at +0.5 V obtained after: (●) ODN modification of GCE and immobilization of ODN 1, (○) hybridization with ODN 2 and (●) control experiments with ODN 3.

The capacitive behaviour of the system can be more conveniently represented using the capacitance data obtained from the impedance spectra.

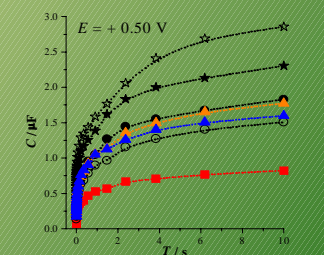


Figure 6 - Capacitance data from impedance spectra in pH 4.5 0.1 M acetate buffer at +0.5 V obtained after: (●) free adsorption of ODN 1 for 7 min, (○) hybridization with ODN 2, (●) ODN 1 modification of GCE, immobilization of ODN 1 for: (●) 3 min and (○) 5 min, (●) hybridization with ODN 2 for 5 min; control experiments for 5 min: (●) ODN 5 and (○) ODN 7.

The impedance spectra demonstrate that at an ODN-modified GCE non-specific adsorption is considerably reduced. However, both selective and non-selective binding to immobilized ODN layers depended on the local environment of the immobilized ODN.