

Detection of DNA Sequences of Bacteria Using a Direct Electrochemiluminescence Method

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A new methodology has been developed which aims at real-time detection of bacteria in drinking water. The DNA sequences of the bacteria are extracted and labeled. The labeled DNA is detected by using a direct electrochemiluminescent (ECL) process on carbon or diamond electrodes.

Real-time detection of microbiological contamination in water is a challenging issue in comparison to the state-of-the-art required minimum test-duration of 48 to 72 hours. Pathogen bacteria in drinking water, like Escherichia Coli, can be detected by identification of their DNA. First, the specific sequences of the DNA have to be selected by hybridization with a corresponding probe. The hybridized product can then be detected. The identification is realized by using the following process:

- Extraction of the DNA of bacteria (all types)
- Denaturation of DNA
- Labeling of single strands
- Selection of DNA by hybridization with a complementary sequence of oligonucleotides of the target bacteria
- Detection and quantification by using the electro-chemiluminescence property of the label.

The ECL process used at CSEM, called "direct ECL" [1], is based on the electronic excitation of the Tris (2,2'-bipyridyl) Ruthenium²⁺ ($Ru(bpy)_3^{2+}$) label which is cycled between oxidizing and reducing electrodes, as shown in Figure 1. As direct ECL asks for a wide range of electrochemical potential, this method is possible only if carbon or diamond electrodes are used.

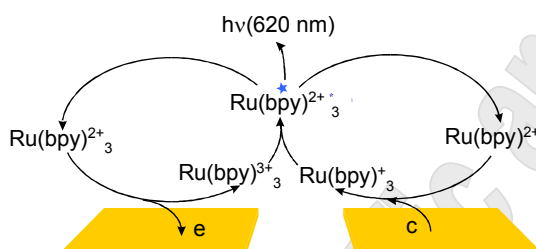


Figure 1: Schematic principle of the direct Electrochemiluminescence

CSEM has built carbon and diamond interdigitated array (IDA) electrodes on silicon chips (Fig. 2). Sequences of oligonucleotides are bonded to the surface of the IDA present on a chip. The chips are placed into a cell for a four channel ECL analysis. The emitted light is guided through four optical fibers and is detected by a photomultiplier. Solutions of $Ru(bpy)_3$ chloride in 10 mM phosphate buffer (PBS) were measured. Results obtained by using a carbon IDA are presented in Figure 3. Over the range of the $Ru(bpy)_3^{2+}$ concentration of 10^{-3} to 10^{-9} M, the log of the peak light-intensity is found to vary linearly with respect to the log of the concentration.

In order to test the method, simplified oligonucleotides sequences have been synthesized. 30 mer-Poly-A sequences with amino groups on 3' have been immobilized on the carbon surface by using the Optodex® technology [2]. 30 mer-Poly-T sequences with amino groups on 3' have been labeled with the Tris(2,2'-bipyridine)-(5-isothiocyanatophenanthroline)ruthenium

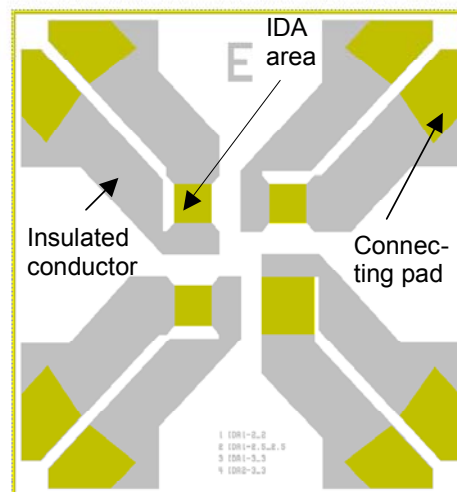


Figure 2: Structure of a multi-channel carbon IDA chip

bis(hexafluorophosphate). After hybridization of Poly-A and Poly-T at room temperature during two hours and washing, the direct ECL detection is performed in PBS solution. The results corresponding to $8 \cdot 10^{-11}$ moles of poly-A /mm² are shown in Figure 3. The light intensity corresponds to a micro-molar solution of $Ru(bpy)_3$ free in solution.

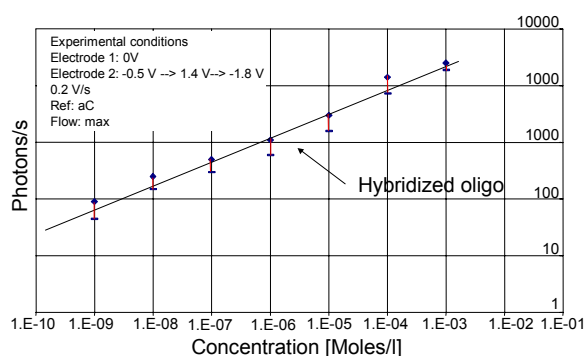


Figure 3: Calibration curve of $Ru(bpy)_3$ free in PBS solution obtained by using a carbon IDA. The light intensity value measured for the hybridized oligo Poly-T is also reported.

This work is a basis for further developments to perform a multi-channel analysis on a chip for the detection of sequences of bacteria DNA. This work was funded by OFES. We thank them for their support.

[1] G.C. Fiaccabrino, M. Koudelka-Hep, Y. Hsueh, S.D. Collins, R.L. Smith, "Electrochemiluminescence of Tris(2,2'-bipyridine) Ruthenium in Water at Carbon Microelectrodes", Analytical Chemistry, 70 (1998) 19

[2] H. Gao, I. Caelen, S. Guinchard, H. Sigrist, "BioArrays photoliés", BIOforum International, Mars 2002, 26