



Electroanalytical quantification of total dsDNA extracted from human sample using, an ionic liquid modified, carbon nanotubes paste electrode



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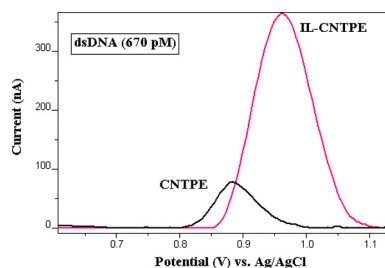
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HIGHLIGHTS

- A paste electrode was prepared by mixing carbon nanotubes with an ionic liquid.
- The electrode was used to investigate the direct electrochemical behavior of dsDNA.
- The presence of IL greatly enhances the peak currents of guanine and adenine.
- dsDNA extracted from human buccal swabs was quantified using the new electrode.
- Detection limit of dsDNA is 16 pM, by applying differential pulse voltammetry.

GRAPHICAL ABSTRACT



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ABSTRACT

Direct electrochemistry of dsDNA has been achieved by using an ionic liquid 1-butyl-4-methylpyridinium hexafluorophosphate modified carbon nanotubes paste electrode (IL-CNTPE). Oxidation peaks appeared at 0.93 and 1.26 V (vs. Ag/AgCl) on the IL-CNTPE after preconcentration of dsDNA in pH 5.0 acetate buffer, which were attributed to the oxidation of guanine and adenine residues on the dsDNA molecule structure. Based on the signal of guanine, under the optimal conditions, very low levels of dsDNA can be detected after 60 s accumulation with detection limits of 0.249 mg L^{-1} 16 pM. Additionally, human DNA from a healthy volunteer is determined by use of the IL-CNTPE and it is found to be $40 \pm 2, 14 \text{ pM}$.

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1. Introduction

Since Paleček discovered the electrochemical activity of nucleic acids, a wide scale research into electrochemistry of natural and chemically modified nucleic acids has started [1–3]. This research have led to a series of voltammetric approach for rapid and inexpensive assays of nucleic acids concentration and structure changes.

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The direct electrochemical behaviors of nucleic acids on different solid electrodes such as glassy carbon electrode, graphite electrode, pencil electrode, or modified electrode was well documented in the literature [4–11]. However, oxidation of adenine and guanine residues on the bare working electrodes exhibited slow direct electron transfer with low sensitivity for nucleic acids detection [12]. Recently, a number of papers were reported on the subject of the direct electrochemistry of nucleic acids using different chemical modified electrodes. Teh et al. fabricated a sol–gel carbon composite electrode in order to study the electrochemistry of adenine. This new electrode was also useful for the simultaneous analysis of adenine and guanine from denatured DNA [13]. Huang et al. described a sodium montmorillonite-modified carbon paste electrode (CPE) for the electrochemical determination of guanine [14]. A fullerene- C_{60} -modified glassy carbon electrode used by Goyal et al. for the simultaneous determination of adenosine and guanosine in human blood plasma and urine [15]. Direct electrochemistry of DNA was observed by Wu et al. at a nanostructure film-modified electrode and a sensitive electrochemical technique for the measurement of native DNA was developed [16].

Recently, carbon nanotubes (CNTs) have attracted much attention as analytical tools, for a wide range of applications, due to their high surface area, good conductance, favorable electronic properties, and electrocatalytic effect [17]. The electrooxidation of guanine and adenine, oligonucleotides, and polynucleotides were also investigated on carbon nanotubes composite materials modified electrodes to study [18–21].

Because of the low solubility of CNTs in most common solvents, the development of paste electrodes is a suitable way to use confined CNTs in electrochemical transducers, using mineral oil, Teflon, ionic liquids (ILs), or epoxy resin as the binder [22].

Ionic liquids (ILs), which are ionic compounds composed of organic cations and various anions, have begun to be used widely in the field of electrochemistry [23]. They show great potential applications in electrochemistry and electrochemical sensors due to the specific characteristics such as wide electrochemical windows, high chemical and thermal stability, relatively high ionic conductivity, negligible vapor pressure [24]. Due to their specific characteristics, ILs can be used as the electrolytes, the modifier on chemically modified electrode and mixed with graphite powder to obtain a new kind of chemically modified carbon paste electrode denoted as carbon ionic liquid electrode (CILE), which exhibits higher performance than the traditional working electrodes. Thus CILE has been used for the detection of different electroactive molecules [24–32] or as a basal electrode for further modification [33,34,26,35–46]. Sun et al. [47,48] studied the electrochemical oxidation of thermally denatured single-stranded DNA (ssDNA), guanine and adenine on a room temperature ionic liquid *N*-butylpyridinium hexafluorophosphate (BPPF₆) modified CILE. Using the same working electrode, the previous group [49] investigated the electrochemical behavior of guanosine and established a new method for its detection. Also, they fabricated [50] a carbon ionic liquid electrode (CILE) by using a hydrophilic ionic liquid 1-ethyl-3-methylimidazolium tetrafluoroborate (EMIMBF₄) as modifier and further covered with multi-walled carbon nanotubes (MWCNTs) to get a modified electrode in order to study the same biomolecule. A hydroxyl functionalized ionic liquid (IL) 1-(3-chloro-2-hydroxy-propyl)-pyridinium acetate was used by Gao et al. [51] as the binder for the preparation of CILE and was further used for the voltammetric determination of adenosine-5'-monophosphate (5'-AMP). Zhu et al. used an ionic liquid (IL) and double-stranded DNA (dsDNA) composite material to investigate the direct electron transfer of myoglobin (Mb) on a carbon ionic liquid electrode (CILE) [23]. Zhang et al. [52] prepared a paste electrode (SWNT&RTIL PE) by using carboxylic group-functionalized short single-walled carbon nanotubes (SWNTs) mixed with

1-butyl-3-methylimidazolium hexafluorophosphate. Based on the current response of guanine bases, the SWNT&RTIL PE showed high sensitivity, reliability, stability, and reproducibility for the detection of oligonucleotides.

In this paper, a carbon nanotubes paste electrode based on the use of 1-butyl-4-methylpyridinium hexafluorophosphate as a binder was prepared and used to investigate the direct electrochemical behavior of dsDNA. Following the oxidation peak of guanine, as the analytical signal, this novel electrode in combination with differential pulse adsorptive voltammetry exhibited very good sensitivity.

2. Experimental

2.1. Reagents

All the chemicals used were reagents of analytical grade. 1-butyl-4-methylpyridinium hexafluorophosphate and double stranded (ds) calf thymus DNA (D-1501, highly polymerized) were purchased from Sigma.

The multi-walled carbon nanotubes (MWNTs) were purchased from Aldrich (Diam. 110–170 nm, length 5–9 μ m, >90% purity).

The dsDNA stock solution (1000 mg L⁻¹) was prepared in 10 mM Tris-HCl and 1 mM EDTA at pH 8.0 [53].

Buccal swabs obtained from a healthy volunteer were used as the source of DNA. Prior to sampling, the volunteer signed an informed consent form.

DNA was extracted from buccal swabs with the use of the QIAampR DNA Mini and Blood Mini Kit (Qiagen).

The concentration of DNA was determined by absorption spectrometry, using the extinction coefficients $\epsilon_{260} = 6600 \text{ M cm}^{-1}$.

2.2. Apparatus

Voltammetric measurements were performed with a PalmSens potentiostat purchased from IVIUM Technologies (The Netherlands, www.ivium.nl) and PalmSensPC software. The working electrode was an ionic liquid modified carbon nanotube paste electrode (IL-CNTPE) with 3 mm inner and 9 mm outer diameter of the PTFE sleeve. The reference electrode was a saturated Ag/AgCl and the counter electrode was a platinum wire.

2.3. Preparation of the working electrodes

The IL-CNTPE was prepared by hand-mixing, in an agata mortar, carbon nanotubes with the ionic liquid form homogeneous mixture and further heated at 60 °C for about 1 h. The content of MWNTs of the electrodes was varied from 20 to 80% and the best performance was obtained at 70% for MWNTs. So the ratio of MWNTs to ionic liquid was 70:30 (w/w) for all the reported experiments.

The carbon nanotube paste electrode (CNTPE) was prepared by mixing in an agata mortar multi-walled carbon nanotubes powder and mineral oil (mass ratio 45:55) to form a homogeneous mixture.

The resulting pastes were packed tightly into a PTFE sleeve and the electrode surface was polished to a smooth finish on a piece of weighing paper. Electrical contact was established with a stainless steel screw. The constructed electrodes were washed with distilled water and then were transferred to a cell containing the measurement solution.

2.4. Procedure

2.4.1. 1L-CNTPE

The study was performed in acetate buffer pH 5.0–5.8, phosphate buffer pH 6.0–7.8, and Tris-EDTA pH 8.0. The studied

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