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# Microanalysis of DNA by stripping transfer voltammetry

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**Abstract**

A cathodic stripping transfer voltammetric procedure for trace determination of DNA and its components is described. The method is based on the DNA acid hydrolysis with subsequent electrochemical determination of released purine bases. In the first step, DNA is hydrolyzed for 30 min in 0.5 M perchloric acid at 75 °C. The electrochemical step involves generation of Cu(I)–purine base complex on a mercury electrode surface, transfer of electrode with accumulated complex into supporting electrolyte where voltammetric measurement is performed. Analysis is carried out in 14-μl drop volume (two-electrode connection) or in 30-μl drop (three-electrode connection) on a platinum plate, which is used as a counter electrode. Blank electrolyte contains 0.05 M borate buffer, pH 9.2 with 6.3 μM Cu(II). We could observe voltammetric signal at hydrolyzed nucleosides, nucleotides, ODN, and DNA containing purine bases. We are able to accumulate under the controlled potential and determine subnanomolar concentration of DNA corresponding to the amount of 200 pg of DNA.


*Keywords:* DNA determination; Determination of purine bases; Linear sweep voltammetry; Electrochemical transfer technique; Hanging mercury electrode; Copper

## 1. Introduction

The interest in determination of DNA has rapidly increased due to huge progress in the development of the electrochemical DNA hybridization sensors. Usually solid electrodes are preferred in these studies, but recently high sensitivity in detection of DNA has been achieved by using cathodic stripping voltammetry (CSV) at mercury or mercury amalgam electrodes for the determination of purine bases, released from DNA by acid pretreatment [1,2]. Applications of electrochemical methods to the determination and hybridization of DNA or oligodeoxynucleotides (ODN) have been reviewed by several authors [3–6].

CSV of nucleic acid bases is based on the formation of sparingly soluble compounds with the electrode mercury, and represents a very sensitive technique [3,7], which was earlier used for determination of nucleic acids bases [8–10]. Glodowski et al. [8] presented determination of traces of purine by CSV at the hanging copper amalgam drop electrode and showed that purine is accumulated on the electrode surface as a Cu(I)–purine complex. Quite recently, Farias et al. [11] used this method for sensitive determination of some nucleic acids bases in presence of Cu(II) in combination of mercury electrode to their ultratrace determination.

In this study, we introduce a simple and sensitive method for the electrochemical determination of DNA or its components containing purine bases. Our approach is based on the similar principle as mentioned above, that is on the formation of the Cu(I)–purine bases complexes at the mercury electrode, but we suggest CSV determination in small volumes using the transfer electrode (medium exchange) technique.

## 2. Experimental

### 2.1. Electrochemical measurements

Linear sweep voltammetry (LSV) measurements at hanging mercury drop electrode (HMDE) were performed with an AUTOLAB analyzer (EcoChemie, The Netherlands) in connection with the VA-Stand 663 (Metrohm, Herisau, Switzerland). The standard cell with two electrodes (2ES) or three electrode (3ES) was used. The working electrode was HMDE (drop area 0.41 $mm^2$), reference electrode was Ag/AgCl/KCl electrode, and platinum plate (1 $cm^2$) was used as the counter electrode. Background electrolyte was 0.05 M borax with 6.3 μM Cu(II). As inert gas, for deaerating solutions in the electrolytic cell, pure argon was used.

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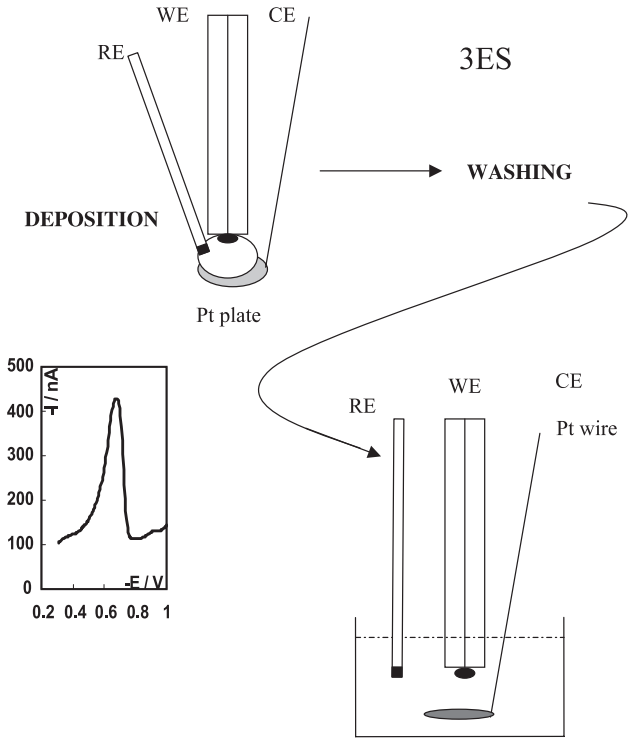


Fig. 1. Scheme of transfer voltammetric technique in three-electrode (3ES) mode. Active material is deposited on the electrode surface under the given potential from a small sample drop (30 μl) of sample. Then the electrode system is transferred through washing step to electrochemical cell containing background electrolyte where the measurement is performed. Resulting voltammogram is shown in insert.

### 2.2. Chemicals

Guanine, adenine, cytosine, thymine, adenosine, guanosine, cytidine, adenylic acid, guanylic acid and cytidylic acid were purchased from Sigma (St. Louis, MO, USA). Oligodeoxynucleotides (ODN) $(A)_{80}$ and $(A)_{25}(GAA)_{24}$ used in this study were synthesized by VBC-Genomics (Wien, Austria). Fragment DNA was prepared from plasmid pBS (2961 bp) by enzymatic digestion and purified on the Microcon YM-30 columns from Millipore. The nucleic acid concentration was determined spectrophotometrically using a HP 8452 spectrophotometer.

### 2.3. Hydrolysis of DNA, ODN, nucleosides and nucleotides

Hydrolysis was performed by adding 20 μl of 1 M $HClO_4$ to sample of the same volume of hydrolyzed sample at concentration of 4 μM (related to the monomer) and heating for 30 min at 75 °C. The sample was cooled down, neutralized with NaOH and aliquots were mixed with background electrolyte and used for voltammetry measurements.

## 3. Results and discussion

### 3.1. Cathodic stripping transfer voltammetry

Earlier we showed that adenine and some other nucleic acid bases form sparingly soluble compounds with the

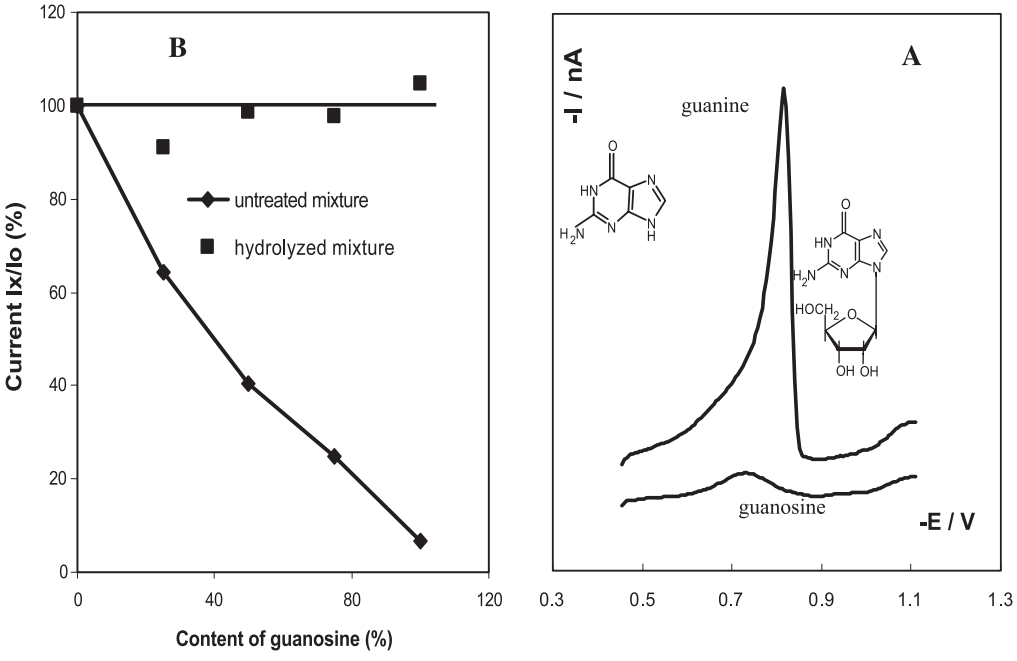


Fig. 2. (A) LS voltammograms of 100 nM guanine and guanosine obtained by transfer technique (CSTV). Guanosine differs from guanine by sugar attached to N(9) of the purine ring. Sugar attachment results in large difference in CSV or CSTV signal in presence of Cu(II). (B) Dependence of voltammetric response on guanosine content in solution. We prepared several samples of guanine–guanosine mixtures with different content of guanosine. The samples contained different ratios of guanine/guanosine, but the total molar concentration was the same. LSV, 3ES, scan rate 0.5 V/s, accumulation time 120 s, deposition potential −0.3 V, 0.05 M borax, pH 9.2 with 6.2 μM Cu(II).

Table 1
Voltammetric signals obtained with some untreated or acid-treated DNA, ODN, nucleosides, nucleotides and nucleic acid base

| Compound | Untreated | Hydrolyzed |
|---|---|---|
| *DNA bases* | | |
| Adenine | + | + |
| Guanine | + | + |
| Cytosine | – | – |
| Thymine | – | – |
| *Nucleosides* | | |
| Adenosine | – | + |
| Guanosine | – | + |
| Cytidine | – | – |
| *Nucleotides* | | |
| Adenylic acid | – | + |
| Guanylic acid | – | + |
| Cytidylic acid | – | – |
| *Oligonucleotide* | | |
| $A_{25}(GAA)_{24}$ | – | + |
| *DNA fragment* | | |
| dsDNA (3000 bp) | – | + |

+: voltammetric signal was observed; –: no voltammetric signal.

electrode mercury and can be determined by CSV after transfer of HMDE (CSTV) to the background electrolyte [12]. We found differences between CSTV behavior of purine and pyrimidine DNA bases and showed that it was necessary to carefully control deposition potential. Recently, we showed that in the analysis of acid-treated DNA in the presence of Cu(II) the accumulation step can be performed at an open current circuit with small volume of acid-treated DNA [1]. The released bases resulting from acid depurination can be quantified by CSV technique. Similar approach we used in this study using simple device, which could accumulate purine–Cu(I) complex under a controlled potential either in two-electrode (2ES) or three-electrode (3ES) mode. Scheme of the 3ES system arrangement is shown in Fig. 1. We performed deposition in 14- or 30-μl drops in 2ES or 3ES mode, respectively. After deposition, we transferred electrodes into electrolyte in electrochemical cell where usual voltammetric measurement was performed. Usually, we used CV or LSV method and observed signal about −0.65 V (3ES) (Fig. 1).

### 3.2. Nucleosides and nucleotides

Nucleosides and nucleotides differ from nucleic acid bases by sugar or sugar-phosphate attached to N(9) of the purine ring or to N(3) of the pyrimidine ring. Sugar or sugar-phosphate attachment results in a large difference in CSV or CSTV signal in presence of Cu(II). For instance, voltammetric signal of guanine compared to guanosine is much higher (Fig. 2A) under comparable

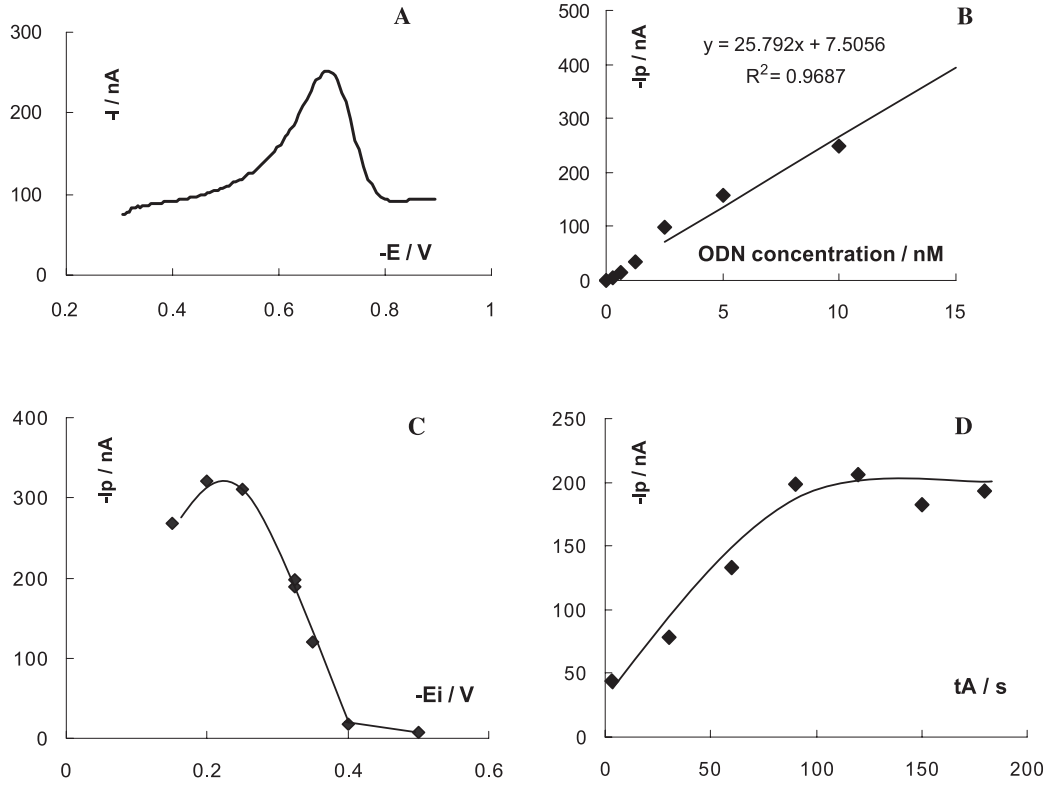


Fig. 3. (A) LS voltammogram of 5 nM ODN $(A)_{25}(GAA)_{24}$. (B) Calibration curve for ODN $(A)_{25}(GAA)_{24}$. Accumulation time 120 s, deposition potential −0.3 V. (C) Dependence of peak height of 5 nM ODN $(A)_{25}(GAA)_{24}$ on deposition potential. (D) Dependence of peak height of 5 nM ODN $(A)_{25}(GAA)_{24}$ on accumulation time. All measurements were obtained by transfer technique. Other conditions as in Fig. 2.

conditions. The results obtained with some other bases, nucleosides and nucleotides are shown in Table 1. The table shows pyrimidine nucleosides and nucleotides after hydrolysis, and pyrimidine bases produce no CSV signal. This may be potentially useful in analysis of purine bases in access of pyrimidines; more details will be published elsewhere.

We prepared several samples of guanine–guanosine mixtures with different content of guanosine to show how would be possible to determine these compounds in mixtures. The samples contained different ratios of guanine/guanosine, but the total molar concentration was the same. When we measured CSTV signal of untreated samples, we observed decrease of signal with increasing amount of guanosine. On the other hand, when the samples were acid treated by perchloric acid, the voltammetric signal was independent on guanosine content (Fig. 2B). We show this example as possible application of cathodic stripping voltammetric technique in bioanalytical chemistry.

### 3.3. Oligodeoxynucleotides and DNA

We measured ODN with sequence $(A)_{80}$ and $(A)_{25}(GAA)_{24}$ using the above CSTV. Voltammetric response for 10 nM $(A)_{25}(GAA)_{24}$ in slightly alkaline medium containing Cu(II) is shown in Fig. 3A. Voltammetric signal at about $-0.65$ V was strongly affected by accumulation potential. We observed this signal at accumulation potential between $-0.2$ and $-0.4$ V with maximum around $-0.3$ V (Fig. 3C). We obtained the best results after 120 s of accumulation (Fig. 1D). For longer accumulation time, no dramatic change of response values was observed. At the optimal condition, we measured CTSV dependence on ODN $(A)_{25}(GAA)_{24}$ concentration and constructed a plot of peak height dependence vs. ODN concentration. We obtained straight line with slope of 27.7 nA/nM with regression coefficient 0.97 (Fig. 3B). We obtained similar results with ODN $(A)_{80}$ and a fragment of plasmid DNA (2961 bp) (not shown).

## 4. Conclusion

In this paper, we propose a new technique for voltammetric determination of acid-treated nucleosides, nucleotides, ODN, which are accumulated at HMDE from small sample volumes under controlled potential. By this technique, determination of subnanogram amounts of DNA and ODNs is possible. The technique can be useful in bioanalytical biochemistry and in the development of DNA sensors.

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