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## Polarographic determination of DNA based on its interaction with the phenanthroline–zinc(II) complex

NI HUI<sup>1\*</sup>, AIQIN LIANG<sup>1</sup>, CHANGHUI XUE<sup>1</sup> and WEI SUN<sup>2</sup>

<sup>1</sup>College of Chemistry and Pharmacy, Qingdao Agriculture University, Qingdao 266109, P. R. China and <sup>2</sup>College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, 571158, P. R. China

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**Abstract:** By using the linear sweep voltammetric technique, a phenanthroline (Phen) and zinc(II) (Phen–Zn(II)) complex was used as the electrochemical probe for the determination of double-stranded (ds) DNA. In pH 9.0 Britton–Robinson (B–R) buffer solution, Phen can interact with Zn(II) to form a stable electroactive [Phen–Zn(II)] complex, which had a sensitive second order derivative polarographic reductive peak at  $-1.300$  V (*vs.* SCE). After the addition of dsDNA into a solution of Phen–Zn(II) complex, the reduction peak current decreased with a negative shift of the reduction peak potential and without the appearance of new peaks. The results showed that a new supramolecular complex was formed *via* interaction of the Phen–Zn(II) complex with dsDNA. The conditions of interaction and the electrochemical detection were carefully investigated. Under the optimum conditions, the decrease in the reduction peak current was directly proportional to the dsDNA concentration in the range of  $0.4$ – $18.0$  mg L<sup>-1</sup> with the linear regression equation:  $\Delta I_p''/nA = 349.48 + 84.647(c/\text{mg L}^{-1})$  ( $n = 13$ ,  $\gamma = 0.991$ ) and a determination limit of  $0.20$  mg L<sup>-1</sup> ( $3\sigma$ ). The relative standard deviation (*RSD*) for 10 parallel determinations of  $10.0$  mg L<sup>-1</sup> dsDNA was found to be  $2.03$  %. The method was successfully applied to the detection of synthetic samples with satisfactory results.

**Keywords:** phenanthroline; zinc; DNA; interaction; linear sweep voltammetry.

### INTRODUCTION

The interaction of small molecules with double-stranded (ds) DNA has been widely investigated in recent years because of the specific functions of dsDNA in life processes. Some interactions could damage the structure of dsDNA and influence the replication of dsDNA.<sup>1</sup> In addition, the investigations could be used for the design of new drugs targeted on dsDNA.<sup>2,3</sup> Generally speaking, small molecules, such as dyes,<sup>4–6</sup> drugs<sup>7–9</sup> and metal coordination compounds,<sup>10–13</sup>

\* Corresponding author. E-mail: huini1029@163.com  
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can bind to dsDNA in three ways, *i.e.*, electrostatic binding, intercalation and groove binding.<sup>14–16</sup> The quantitative determination of DNA is also important in bioanalytical chemistry and clinical tests. Various analytical methods for the detection of DNA, such as spectrophotometry,<sup>17–19</sup> fluorometry,<sup>20,21</sup> light scattering technique,<sup>22,23</sup> *etc.*, have been established based on the interaction of small molecules with dsDNA. However, most of these methods suffer from low sensitivity, high costs or complicated procedures. Compared with the above methods, the electroanalytical method has some advantages. Such as cheaper and smaller devices, wider linear range and lower detection limits.<sup>24</sup> In addition, as the electrochemical reaction occurs at the electrode interface, only a small amount of sample is required in the detection procedure. Wang *et al.*<sup>25</sup> established an electrochemical equation for examining the interaction of irreversible redox compounds, such as bis-benzimidazole derivative (Hoechst 33258) with dsDNA using cyclic voltammetry and other electrochemical techniques. Paleček *et al.*<sup>26</sup> studied the interaction between the anticancer drug mitomycin C and DNA by cyclic voltammetry on a hanging mercury drop electrode, which resulted in a decrease in the voltammetric signal. Jiao *et al.* used some organic dyes, such as toluidine blue,<sup>27</sup> rhodamine B<sup>28</sup> and malachite green,<sup>29</sup> for the detection of dsDNA based on the formation of electroinactive complexes on mixing a mixture of dsDNA with the dyes in solution. Bard *et al.*<sup>30,31</sup> reported the interaction of metal chelates of tris (1,10-phenanthroline) cobalt (III) with DNA in an aqueous medium and established a redox current equation for the intercalator–DNA complex. Moreover, some polarographic methods for the detection of dsDNA were established using electroactive dyes, such as pyronine B,<sup>32</sup> phenosafranine,<sup>33</sup> neutral red<sup>34</sup> and brilliant cresyl blue.<sup>35</sup>

Phenanthroline (Phen) is a heterocyclic organic compound that is often used as a bidentate ligand in coordination chemistry. Phen reacts with most metal ions to yield strong stable metal complexes, such as with cobalt(III)<sup>30</sup> and copper(II).<sup>36</sup> It was employed in the determination of zinc(II) by polarography.<sup>37,38</sup> Experimental results indicated that the Phen–Zn(II) complex had a sensitive polarographic reduction peak at  $-1.300$  V (*vs.* SCE) on a dropping mercury working electrode. After the addition of dsDNA into a Phen–Zn(II) complex solution, a large decrease in the reduction peak current and a negative shift of the reductive peak potential appeared, which indicated that Phen–Zn(II) complex could interact with dsDNA to form a supramolecular complex in the mixture solution. In this study, the phenanthroline (Phen) and zinc complex was investigated as a new polarographic probe for dsDNA detection.

## EXPERIMENTAL

### *Apparatus and reagents*

All the polarographic determinations were performed on a model JP-303 polarography (Chengdu Apparatus Factory, China) with the second order derivative linear sweep polaro-

graphy. A conventional three-electrode system, which was composed of a dropping mercury working electrode (DME), a saturated calomel reference electrode (SCE) and a platinum wire auxiliary electrode, was used throughout. A pHs-25 acidimeter (Shanghai Leici Instrument Factory, China) was used for the pH value measurement and all the experiments were performed at  $25 \pm 1$  °C.

A stock solution ( $1.0 \text{ g L}^{-1}$ ) of double-stranded (ds) herring sperm DNA (Sigma, USA) was prepared by dissolving it in doubly distilled water and storing at 4 °C.<sup>39-41</sup> The skeleton of dsDNA is very stable and apart from strong acids and high temperature, other environments cannot destroy it.<sup>42</sup> Experiments showed that the  $A_{260}$  of the dsDNA solution kept in 4 °C did not change, indicating that the low temperature environment prevented it from unwinding. The purity of dsDNA was examined by the absorbance ratio of  $A_{260}/A_{280}$  that was 1.85, which indicated that the dsDNA was sufficiently free of protein. The concentration of dsDNA was calculated from the absorbance value at 260 nm using  $\epsilon_{\text{DNA}} = 6600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ . Single-stranded DNA (ssDNA) was acquired by heating the solution of native herring sperm DNA at 100 °C in a waterbath for 10 min and cooling in an ice-water bath.<sup>41</sup> A  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  phenanthroline (Phen, Shanghai Sanpu Chemical Reagent Factory, China) solution was prepared by dissolving 0.1802 g Phen in water and diluting to 250 mL. Britton–Robinson (B–R) buffers ( $0.2 \text{ mol L}^{-1}$ ) were used to adjust the pH values of test solution. When B–R buffer was adjusted to pH of 9.0, its ionic strength was 0.104.<sup>43</sup> All the other reagents were of analytical reagent grade and doubly distilled water was used throughout.

#### Procedures

Into a dry 10 mL volumetric flask, the following reaction solutions were added in turn: 2.0 mL of  $0.2 \text{ mol L}^{-1}$  B–R buffer solution, 1.0 mL of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  Phen, 0.5 mL of  $1.0 \times 10^{-3} \text{ mol L}^{-1}$  Zn(II) and different amounts of dsDNA solution. The mixtures were diluted to 10 mL with water, mixed thoroughly and allowed to stand for 20 min at 25 °C. Then the solution mixture was transferred to a 10 mL electrochemical cell and the second order derivative linear sweep polarographic curve was recorded in the potential range from  $-0.8$  to  $-1.6$  V (vs. SCE) and the reduction peak current ( $I_p''$ ) of the solution was measured. The peak current of the blank solution ( $I_{p_0}''$ ) without dsDNA was obtained under the same conditions and the difference of peak currents ( $\Delta I_p'' = I_{p_0}'' - I_p''$ ) was used for dsDNA determination.

## RESULT AND DISCUSSION

### *Second order derivative linear sweep polarography*

Typical second order derivative linear sweep voltammograms of the buffer, the Phen–Zn(II) complex and two Phen–Zn(II)–dsDNA reaction solutions are shown in Fig. 1. Curve 1 is the polarogram of the B–R buffer solution without any polarographic peak. Curve 2 is the polarogram of the Phen–Zn(II) complex solution with a reduction peak at  $-1.300$  V (vs. SCE), which is the typical Phen–Zn(II) wave on a mercury electrode. The formation and the electrochemical behavior of the [Phen–Zn(II)] complex were explained in detail in the literature.<sup>37,38</sup> Curves 3 and 4 are polarograms of the mixture containing different amounts of dsDNA and the Phen–Zn(II) complex. It can be seen that the reduction peak current decreased gradually with the negative shift of the reduction peak potential, which indicated electrostatic binding of the Phen–Zn(II) complex with dsDNA to form a supramolecular complex. The decrease in the peak current

was proportional to the concentration of dsDNA, which could be further used for the determination of dsDNA.

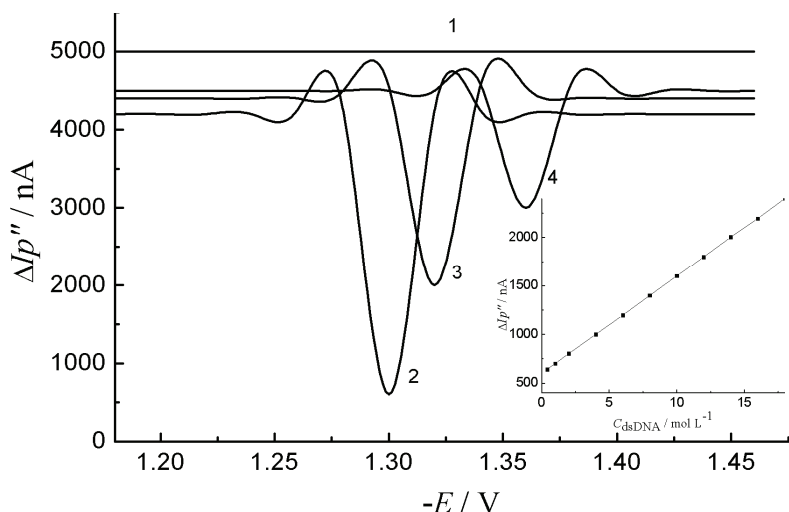


Fig. 1. Second order derivative linear sweep voltammograms of the Phen-Zn(II)-dsDNA reaction system. Inset: the linear calibration curve of dsDNA. 1: pH 9.5, B-R buffer; 2: 1 +  $1.0 \times 10^{-4}$  mol L $^{-1}$  Phen +  $5.0 \times 10^{-5}$  mol L $^{-1}$  Zn(II); 3: 2 + 8.0 mg L $^{-1}$  dsDNA; 4: 2 + 18.0 mg L $^{-1}$  dsDNA.

#### Optimization of the reaction conditions

The effect of buffer acidity on the difference in the peak current ( $\Delta I_p''$ ) was investigated in the pH range between 7.0 and 12.0 and the results are shown in Fig. 2. It could be seen that the value of  $\Delta I_p''$  reached its maximum at pH 9.0; hence, pH 9.0 was selected for this assay. The volume of B-R buffer solution added into the solution was also investigated and 2.0 mL of B-R buffer solution was suitable as the supporting electrolyte having sufficient buffer capacity.

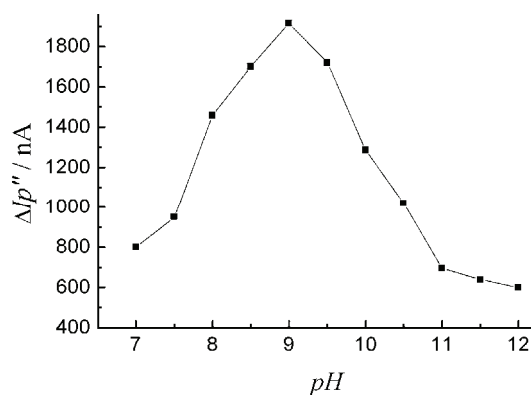


Fig. 2. The influence of pH on the binding interaction.  $1.0 \times 10^{-4}$  mol L $^{-1}$  Zn(II) +  $1.0 \times 10^{-4}$  mol L $^{-1}$  Phen + 10.0 mg L $^{-1}$  dsDNA in B-R buffers of different pH values.

The effect of the mole ratio of Phen and Zn(II) on the value of  $\Delta I_p''$  was studied by fixing the dsDNA concentration at  $10.0 \text{ mg L}^{-1}$ , the Zn(II) concentration at  $5.0 \times 10^{-5} \text{ mol L}^{-1}$  and changing the concentration of Phen. The value of  $\Delta I_p''$  reached a maximum when the Phen concentration was  $1.0 \times 10^{-4} \text{ mol L}^{-1}$ . Hence, the ratio of Phen to Zn(II) was kept at 2:1 in the following experiments.

After mixing Phen, Zn(II) with dsDNA in the solution, the value of  $\Delta I_p''$  reached a maximum within 20 min and remained constant for 2 h. Hence, an incubation time of 20 min was used in the experiments, and thus the system gave sufficient time for routine measurements.

The influence of the order of the additions of the different solution on the value of  $\Delta I_p''$  was also studied. The optimal addition order was selected as B–R, Phen, Zn(II) and dsDNA. The results indicated that in the selected pH buffer, the formation of Phen–Zn(II) complex was crucial to the interaction.

The effect of the reaction temperature on the interaction was also tested in the range of 10–40 °C and no obviously differences on  $\Delta I_p''$  was found in the selected temperature range. Thus, a reaction temperature of 25 °C was used throughout.

The effect of instrumental conditions such as the scan rate and the mercury dropping time were carefully tested in turn. The results expressed that the value of  $\Delta I_p''$  increased with the increase of potential scan rate in the range of 300 to 1000  $\text{mV s}^{-1}$ . Thus, 1000  $\text{mV s}^{-1}$  was selected as the scan rate for detection. The dropping mercury developing time for the assay was optimized and selected as 8 s.

#### *Effect of ionic strength*

The effect of ionic strength on the interaction was also investigated by the addition of different amounts of  $0.10 \text{ mol L}^{-1}$  NaCl to the mixture. The value of  $\Delta I_p''$  decreased with increasing salt concentration in the solution, indicating that the ionic strength had significant influence on the interaction. Thus, the interaction of Phen–Zn(II) complex with dsDNA was caused by a highly favored electrostatic attraction. The addition of NaCl to the reaction solution could weaken the electrostatic force between the Phen–Zn(II) complex and dsDNA.

#### *Effect of ssDNA and dsDNA on peak current*

The effect of ssDNA and dsDNA on the change of reduction peak current was further investigated. It could be seen that both ssDNA and dsDNA can cause a decrease in the reduction peak current, but that ssDNA had a smaller effect on the peak current than did dsDNA. The results indicated that the Phen–Zn(II) complex could interact with phosphate groups outside the DNA strand by electrostatic interaction and the fact that there are more phosphate groups on dsDNA than on ssDNA resulted in a great decrease in the reduction peak currents.

### Influences of coexisting substances

The influences of co-existing substances on the determination of 12.0 mg L<sup>-1</sup> dsDNA were also tested and the results are listed in Table I. It could be seen that most of the compounds did not disturb the determination. However, some metal ions, such as Al<sup>3+</sup> and Co<sup>2+</sup>, exhibited some influence, which may be due to the formation of metal complex with Phen.

TABLE I. Influence of co-existing substances on the determination of 12.0 mg L<sup>-1</sup> dsDNA

Co-existing substance, 2.0 mg L <sup>-1</sup>	Relative error, %	Co-existing substance, 5.0 μmol L <sup>-1</sup>	Relative error, %
L-Glutamine	3.23	Ca <sup>2+</sup>	-1.94
L-Tyrosine	5.02	Mg <sup>2+</sup>	0.65
L-Cystine HCl monohydrate	1.29	Mn <sup>2+</sup>	5.00
L-Arginine	4.34	Al <sup>3+</sup>	8.48
L-Leucine	2.20	Co <sup>2+</sup>	9.78
L-Cysteine	10.4	Pb <sup>2+</sup>	1.48
Glycine	3.92	β-CD	0.00
Glucose	3.91	SDS	-5.63
L-Glutamic acid	-3.23		

### Calibration curve

Under the optimal conditions, the decrease of reduction peak current was proportional to the concentration of dsDNA in the range from 0.4 to 16.0 mg L<sup>-1</sup> with the linear regression equation:

$$\Delta I_p''/nA = 349.48 + 84.647(c / \text{mg L}^{-1})$$

$$(n = 13, \gamma = 0.991)$$

The *RSD* of 10 parallel determinations of 10.0 mg L<sup>-1</sup> dsDNA was 2.03 %, indicating the good reproducibility of the detection. The limit of detection (*LOD*) for dsDNA was calculated using the equation:

$$LOD = 3S_0/S$$

where 3 is the factor at the 99 % confidence level, *S*<sub>0</sub> is the standard deviation of the blank measurement, and *S* is the slope of the calibration curve. The *LOD* was calculated to be 0.20 mg L<sup>-1</sup>

The proposed method is compared with other methods in Table II. It could be concluded that although the sensitivity of this method was not comparable to

TABLE II. Comparison of the analytical results for the determination of DNA with those in the literature

Method	Linear range, mg L <sup>-1</sup>	<i>LOD</i> / mg L <sup>-1</sup>	Ref.
Light-scattering technique	0.112–4.64	0.112	44
Fluorometry	0.02–0.80	0.007	45
LSV at DME	0.4–16.0	0.198	This paper

that of the light-scattering technique and fluorometry, the widest linear range with good selectivity made this method more practical for routine tests.

#### Sample determinations

Three synthetic samples containing some metal ions, amino acids, *etc.* were analyzed by the proposed method with the standard addition method and the obtained results are listed in Table III. A good recovery was obtained in the range of 95.1–99.5 %, indicating that this new method was practical and reliable for the determination of dsDNA in synthetic samples.

TABLE III. Determination results of dsDNA in synthetic samples ( $n = 5$ ); conditions: L-leucine, L-arginine, L-tyrosine, glycine, L-glutamine, L-glutamic acid glucose,  $2.0 \text{ mg L}^{-1}$ ;  $\text{Ca}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Al}^{3+}$ ,  $\text{Mg}^{2+}$ ,  $\beta\text{-CD}$ ,  $5 \times 10^{-6} \text{ mol L}^{-1}$

No.	Foreign co-existing substances	dsDNA, $\text{mg L}^{-1}$		Recovery %	RSD %
		Added	Found		
1	$\text{Ca}^{2+}$ , $\text{Mg}^{2+}$ , L-arginine, L-tyrosine	10.00	9.71	97.1	2.12
2	$\text{Pb}^{2+}$ , $\text{Ca}^{2+}$ , L-teucine, glycine, $\beta\text{-CD}$	10.00	9.95	99.5	1.02
3	$\text{Mn}^{2+}$ , $\text{Al}^{3+}$ , glucose, L-glutamine, L-glutamic acid	10.00	9.51	95.1	3.20

#### Measurement of the binding ratio

The binding ratio of Phen–Zn(II) complex with dsDNA was calculated by the commonly used mole ratio method. By keeping the Phen–Zn(II) complex concentration constant at  $4.5 \times 10^{-5} \text{ mol L}^{-1}$  and changing the dsDNA concentration, the relationship between  $I_{p''}$  and  $c_{\text{dsDNA}}$  was obtained and is plotted in Fig. 3. It can be seen that when the concentration of dsDNA was higher than  $18.0 \text{ mg L}^{-1}$ , the reduction peak current remained stable, indicating that the interaction had reached its equilibrium. Then the intersection point of the two linear curves was obtained as  $14.61 \text{ mg L}^{-1}$  ( $4.48 \times 10^{-5} \text{ mol L}^{-1}$ ) dsDNA. Thus, the stoichiometry of Phen–Zn(II) complex with dsDNA was calculated as 1:1, which indicated that a Phen–Zn(II)–dsDNA supramolecular complex was formed under the selected conditions.

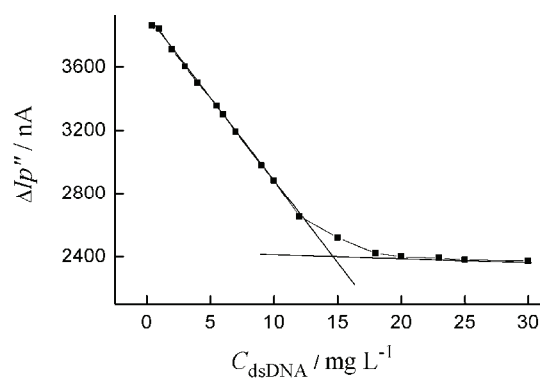


Fig. 4. Determination of the coordination number by molar ratio method pH 9.0 B–R buffer +  $4.5 \times 10^{-5} \text{ mol L}^{-1}$  Phen–Zn(II) complex with different amounts of dsDNA.



## CONCLUSIONS

A new electroanalytical method for the determination of dsDNA using the Phen–Zn(II) complex was investigated in this study. On addition of dsDNA to a solution of the Phen–Zn(II) complex, a decrease in the reduction peak current occurred, which could be employed for the micro-determination of dsDNA. Under the selected conditions, a new supramolecular complex was formed by electrostatic binding and the binding ratio of Phen–Zn(II) complex with dsDNA was calculated by the mole ratio method to be 1:1. Under the optimal conditions, dsDNA could be detected in the concentration range from 0.4 to 16.0 mg L<sup>-1</sup> with a detection limit of 0.20 mg L<sup>-1</sup> (3σ). The proposed method is practical and reliable with a simple procedure and could be used for dsDNA quantification in real samples.

## ИЗВОД

## ПОЛАРОГРАФСКО ОДРЕЂИВАЊЕ ДНК БАЗИРАНО НА ИНТЕРАКЦИЈАМА СА ФЕНАНТРОЛИН–ЦИНК(II) КОМПЛЕКСОМ

NI HUI<sup>1</sup>, AIQIN LIANG<sup>1</sup>, CHANGHUI XUE<sup>1</sup> и WEI SUN<sup>2</sup>

<sup>1</sup>College of Chemistry and Pharmacy, Qingdao Agriculture University, Qingdao 266109, P. R. China и

<sup>2</sup>College of Chemistry and Chemical Engineering, Hainan Normal University, Haikou, 571158, P. R. China

Применом волтаметрије са линеарном променом потенцијала, комплекс фенантролина и цинка(II) (Phen–Zn(II)) је коришћен за електрохемијско испитивање и одређивање ДНК у форми дволанчане спирале (dsDNA) Фенантролин реагује са цинком(II) у Бритон–Робинсоновом пуферу, рН 9,0, градећи стабилан електроактиван Phen–Zn(II) комплекс, који даје поларографски редукциони сигнал (у односу на zasiћену каломелову электроду) и показује осетљив пик добијен као извод другог реда на потенцијалу од –1,300 V. После додатка dsDNA у раствор Phen–Zn(II) комплекса, струја редукционог пика се смањује, уз померање потенцијала пика ка негативнијим вредностима и без појаве нових пикова. Резултати указују на формирање супрамолекуларног комплекса, преко интеракција Phen–Zn(II) комплекса са дволанчаном спиралом ДНК. Испитивани су услови интеракције и електрохемијске детекције. При оптималним условима, смањење струје редукционог пика је директно сразмерно концентрацији dsDNA у опсегу 0,4–18,0 mg L<sup>-1</sup> са регресионом једначином  $\Delta I_p''/nA = 349.48 + 84.647(c / \text{mg L}^{-1})$  ( $n = 13$ ,  $\gamma = 0,991$ ) и границом одређивања од 0,20 mg L<sup>-1</sup> (3σ). Релативна стандардна девијација (RSD) за 10 паралелних одређивања 10,0 mg L<sup>-1</sup> dsDNA износи 2,03 %. Метода је успешно примењена за детекцију синтетичких узорака и добијени су задовољавајући резултати.

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