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Research Article

Resonance Rayleigh Scattering Method for the Determination of Green Food DNA Based on the Interaction of O-hydroxyphenylfluorone with DNA

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Abstract: A new spectroscopic analysis method was built for the determination of green food DNA based on the interaction of o-Hydroxyphenylfluorone (o-HPF) with DNA using the resonance Rayleigh Light Scattering (RLS) technique. In the Tris-HCl buffer at pH 8.1, the RLS signal of o-HPF was enhanced remarkably in the presence of DNA and the enhanced RLS intensity at 519 nm was in direct proportion to DNA concentration in the range of 0.02-1.8×10^{-5} g/L with a good linear relationship. The detection limit was 4.6×10^{-8} g/L. The method was simple, rapid and high accuracy which had been applied to the determination of green food DNA in sample with satisfactory results.

Keywords: Green food DNA, o-HPF, resonance rayleigh light scattering

INTRODUCTION

Nucleic acids are the basic substances of life, which present in all organisms. They play a decisive role in the storage of genetic information and the biosynthesis of protein. Therefore, the qualitative and quantitative analyses of nucleic acids, in particular Double-Stranded (DS) DNA, have been attracted considerable interest because of its significant importance in analytical area. Currently the techniques often applied to the determination of DNA include UV spectroscopy (Duan *et al*., 2007), fluorophotometry (Sabolová *et al*., 2006), radioactive labeling method (Yi *et al*., 2003), electrochemical method (Kang *et al*., 2007), Resonance Rayleigh Light Scattering (RLS) (Li *et al*., 2011a; Bi *et al*., 2012) and others. Among them, the RLS technique has been studied intensively for decades owing to its high sensitivity, rapidity and convenient operation, which was proposed by Pasternack and co-workers (Pasternack *et al*., 1993).

RLS is an elastic scattering that electromagnetic radiation and material act each other and generate the elastic collisions. This kind of scattering was produced as the wavelength of Rayleigh Scattering (RS) is located at or close to its molecular absorption band. Thus, the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. Due to the intensive absorption of light energy of the electron, re-scattering takes place. Therefore, the scattering signal is enhanced several orders of magnitude compared with that of single RS and no longer follows the Rayleigh law of $I\propto\lambda^{-4}$ (Luo *et al*., 2010; Stanton, 1981). RLS displays also the characteristics of the scattering spectrum as well as that of the electronic absorption spectrum. Compared with other analysis technique, RLS technique not only possesses high sensitivity and better selectivity, but also can provide a plenty of useful information concerning molecular structure, size, form, charge, distribution, state of combination and so on. These advantages make RLS technique popular in many research areas including biotechnology (Chen *et al*., 2012), quality inspection (Li *et al*., 2011b), biochemical analysis (Xiao *et al*., 2012), clinical diagnosis (Cui *et al*., 2011) and investigation of dynamic process (Luo *et al*., 2010).

This study is based on the interaction between DNA and triphenylmethane dyes phenylfluorone synthesized in our own laboratory (Huang and Zhang, 2001; Luo *et al*., 2002). Phenylfluorone and its derivatives are a class of spectral analysis reagent with superior performance, which was widely used in the determination of trace metal ions (Amelin Abramenkova, 2008), EDTA (Fujita *et al*., 1998) and so on. In this assay, *o*-Hydroxyphenylfluorone (*o*-HPF) was introduced and a more sensitive probe of DNA was proposed. When calf thymus DNA (ctDNA) was loaded in the solution of *o*-HPF, the RLS intensity of *o*-HPF could be enhanced accordingly and the enhanced intensity was in proportion to the concentration of DNA

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in the range of 0.02-1.8×10^{-5} g/L. The method has better selectivity and can be applied to the direct determination of trace amounts of ctDNA in synthetic samples and practical samples. A simple, convenient method with high sensitivity and efficiency for determination of DNA is established.

MATERIALS AND METHODS

Materials: The RLS spectra were performed on an F-4500 spectrofluorometer (Hitachi, Japan) equipped with a xenon lamp source and a quartz cuvette (1.0×1.0×1.0 cm). The UV-vis spectra were obtained by a 756 PC spectrometer (Shanghai spectrum co., LTD). A WH-2 vortex mixer (Huxi Instrumental Co., Shanghai, China) was used to blend the solution and a PHB-4 pH meter (Leici Instrumental Co., Shanghai, China) was used to measure the pH value of the solution.

Stock solution of ctDNA (100 mg/L) was prepared by dissolving commercially purchased calf thymus DNA (ctDNA, Beijing Xinjingke reagent company) in 100 mL double distilled water. The DNA stock solution was stored at 4°C in the dark for a week.

o-HFP obtained in our own laboratory was used to prepare 1×10^{-3} mol/L stock solution as follows: 0.0336 g of o-hydroxyphenyl fluorone was firstly made by HCl solution (1:1), then transferred into a 100 mL volumetric flask and diluted to volume with anhydrous ethanol. The stock solution was stored in dark and the required working concentration was obtained by diluting the stock concentration with water.

Tris-HCl buffer solution: One hundred and twenty one grams of Tris base was dissolved in about 0.9 L water, then a certain amount of concentrated HCl (11.6 mol/L) was loaded according to the required pH (below 25°C), then and diluted to 1 L with water. A Britton-Robinson (BR) buffer with an ionic strength of 0.5 was prepared from an acidic solution that contained 0.04 mol/L each of H_3PO_4, HOAc and H_3BO_3 by adjusting to appropriate pH using 0.2 mol/L NaOH.

All the other reagents were of analytical reagent grade and were used without further purification. Doubly distilled water was used throughout.

Synthesis of o-hydroxyphenylfluorone: The synthesis, purification and characterization of *o*-hydroxyphenylfluorone was based on our previous study in our own laboratory (Huang and Zhang, 2001; Luo *et al*., 2002).

Experimental methods: Appropriate working solution of DNA were added to a 10 mL volumetric flask, adjusted the pH value to 8.1 using the Tris-HCl solution and then added 2 mL of *o*-HPF solution (1.0×10^{-6} mol/L). The mixture was diluted with water to 10 mL and vortexed for 12 min. After that, all the absorption and RLS measurements were obtained against the blank treated in the same way without DNA. The RLS spectrum was obtained by scanning simultaneously the excitation and emission monochromator of the F-4500 spectrofluorometer at λem = λex (519 nm) and measure the RLS intensity I_{RLS} for the reaction product and I_0 for the reagent blank at the maximum scattered wavelength, $\Delta I = I_{RLS} - I_0$.

RESULTS AND DISCUSSION

Resonance light scattering spectra: The RLS spectral characteristics of *o*-HFP and *o*-HFP-DNA solution with different concentration of DNA at pH 8.1 are shown in Fig. 1. It could be observed that *o*-HFP alone (Curve 1) showed obvious RLS signal in the range of 200-700 nm. As some of DNA solution was introduced to the *o*-HFP system, the RLS intensity was enhanced and the maximum resonance spectra peaks located at 519 nm. We attributed the enhance RLS intensity to the electrostatic interaction between *o*-HPF and DNA that resulted in the formation of the larger *o*-HPF particles, which intensified the RLS signals. We also found that, the enhanced RLS intensity was in direct proportion to the concentration of DNA within a certain range with a good linear relationship.

UV-vis adsorption spectra: The UV-vis adsorption spectra of *o*-HFP and *o*-HFP-DNA solution are shown in Fig. 2. As can be seen clearly, *o*-HFP displayed characteristic adsorption at 476 nm. When interacted with DNA, the characteristic peak red shifted to 497 nm and the intensity was decreased. This suggested that the electrostatic interaction between *o*-HFP and DNA lead to the formation of new complexes in which the charge migration of the negatively charged *o*-HPF molecular became easier than the uncombined one, so the adsorption spectra was red shifted.

Effect of pH and the choice of buffer solution: Here, we used Tris-HCl and Britton-Robinson (BR) solution to control the acidity of the system according to the procedure and the enhanced intensity of RLS in different acidity was shown in Fig. 3. It was clear that, in both buffer solutions, the RLS showed similar variation tendency. In strong acid solution and strong alkaline solution, the enhanced RLS signal was not evident. We assumed that, under strong acidic conditions, the double helix structure of DNA was seriously damaged and the blank value increased which in turn result the reduced ΔRLS. When in an alkaline solution, the protonation of *o*-HPF was not distinct enough to form very stable *o*-HFP-DNA complex, so the ΔRLS was small. At pH 8.1, the scattered light intensity underwent the maximal changes. Compared with in the BR solution, ΔRLS showed much higher intensity than that of in the Tris-HCl buffer solution, which due to the presence of negatively anions in BR

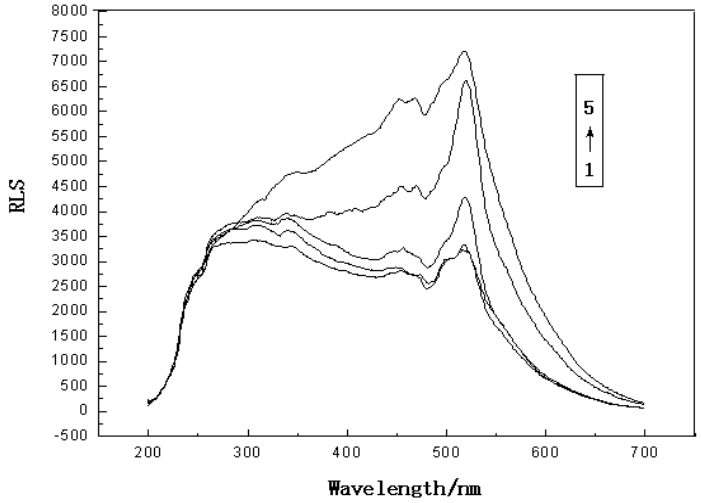

Fig. 1: Resonance Light-Scattering (RLS) spectra of DNA-o-HFP system
pH: 8.1; $c_{o\text{-}HPF}$ 2.5×10^{-7} mol/L; 1: c_{DNA} 0; 2: c_{DNA} 2.5×10^{-6} g/L; 3: c_{DNA} 5.0×10^{-6} g/L; 4: c_{DNA} 7.5×10^{-6} g/L; 5: c_{DNA} 1.0×10^{-5} g/L

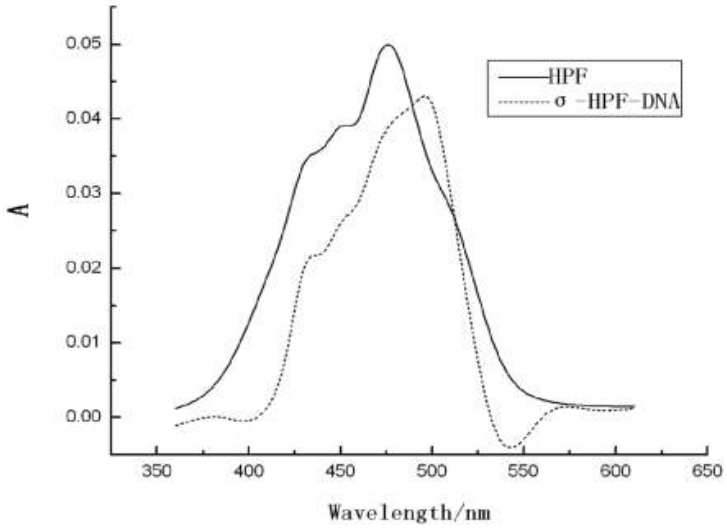

Fig. 2: Absorption spectra of o-HPF and o-HFP-DNA (c_{DNA} 5.0×10^{-6} g/L; $c_{o\text{-}HPF}$ 2.0×10^{-7} mol/L; pH 8.1)

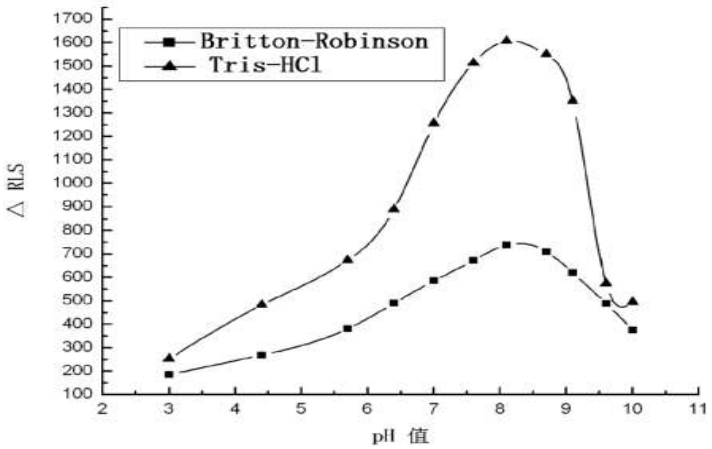

Fig. 3: Effect of pH on the intensity of ΔRLS ($c_{o\text{-}HPF}$ 2×10^{-7} mol/L; c_{DNA} 5.0×10^{-6} g/L)

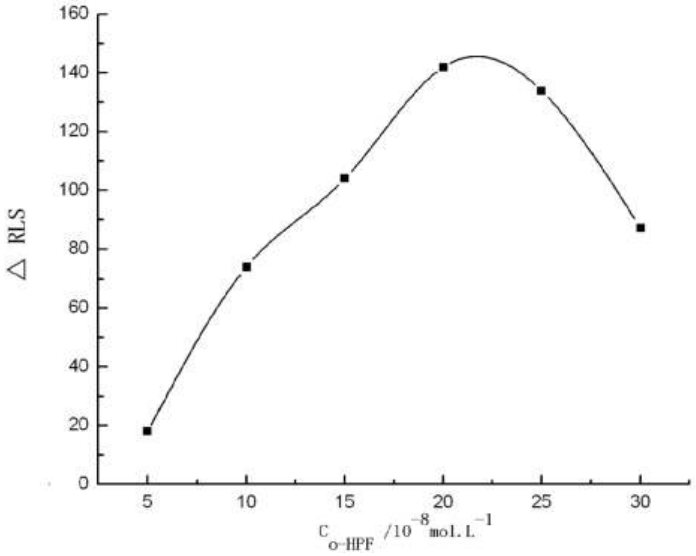

Fig. 4: Effect of o-HPF concentration on the intensity of RLS (pH 8.1; c_{DNA} 5.0×10^{-6} g/L)

solution showed competition reaction with DNA, caused the fewer combination of *o*-HFP with DNA and so the reduced ΔRLS intensity. Thus, the reaction was conducted in the Tris-HCl buffer solution with a pH value of 8.1.

Effect of the amount of o-HFP: The effect of the amount of *o*-HFP used in the system was also investigated and the result was displayed in Fig. 4. Obviously, the ΔRLS intensity was highly relevant to the amount of *o*-HFP. Too much or too little *o*-HFP in the solution would cause ΔRLS intensity decrease. When the *o*-HPF concentration was 2×10^{-7} mol/L, ΔRLS showed the maximum value. As the concentration of *o*-HPF was low, the molecular of *o*-HPF was simply embedded in the base pairs of nucleic acid, which just slightly increased the length and viscosity of DNA. The interaction of DNA and *o*-HFP was not complete, so the increased RLS intensity was not evident. With the increasing concentration *o*-HPF, more and more *o*-HPF molecular gathered on the DNA strand, resulting in a strong resonance spectroscopy, until all the DNA was transformed to the combination product. When the concentration of *o*-HPF increased further, ΔRLS decreased. This was because with the increase in the concentration of *o*-HPF, its scattered light intensity, that is, the blank resonance spectral intensity also increased and finally leads to the reduced ΔRLS intensity.

Effect of reaction time: The effect of the reaction time from 5 to 50 min was discussed and the result was exhibited in Fig. 5. One can see that, in the pH 8.10 of Tris-HCl buffer solution, the reaction of DNA and *o*-HPF was rapid and got stable after 12 min. The formed complex can keep stable in 30 min. Prolonging the reaction time would lead to the reduced ΔRLS value. It was because that the formed complex possessed a hydrophobic surface which would aggregate and precipitate in the solution, so too much time leaded to a low ΔRLS value. Therefore, the reaction time was set as 12 min.

Effect of reaction temperature: The reaction was conducted under different temperature from 10-100°C, respectively and the obtained ΔRLS values were shown in Fig. 6. When the reaction occurred at 35°C, the Δ RLS intensity showed the maximum. Probably in this temperature, the reactant in the system possessed the best activity, especially for the bio-macromolecule DNA, the movement of particle was speeded up, then the combination of *o*-HPF and DNA was tight, which showed highest ΔRLS values. While when the temperature was above 40°C, the structure of DNA was dramatically damage, which results in the poor combination between DNA and *o*-HPF and a decreased ΔRLS intensity. In our study the reaction was optimized at room temperature.

Effect of ionic strength: We picked strong electrolyte NaCl (0.10 mol/L) to adjust the ionic strength of the reaction system and the effect of ionic strength on the ΔRLS intensity was shown in Fig. 7. It could be seen that the introduction of NaCl, regardless of high or low ionic strength, showed little impact on the RLS intensity of *o*-HPF itself. However, the high ionic strength would cause significant decrease ΔRLS intensity. This may be due to the conversion of DNA conformation in the presence of strong electrolyte with high concentration (Sheardy and Winkel, 1989). In

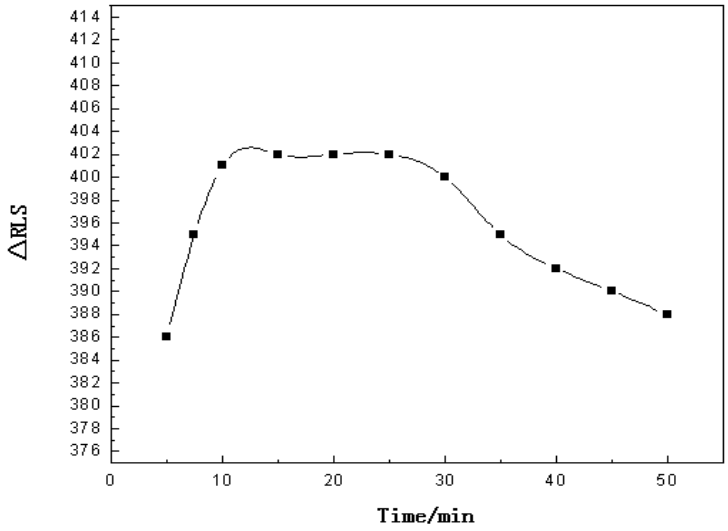

Fig. 5: Effect of time on the intensity of RLS (pH 8.1; c_{DNA} 5.0×10^{-6} g/L; $c_{o\text{-}HPF}$ 2.0×10^{-7} mol/L)

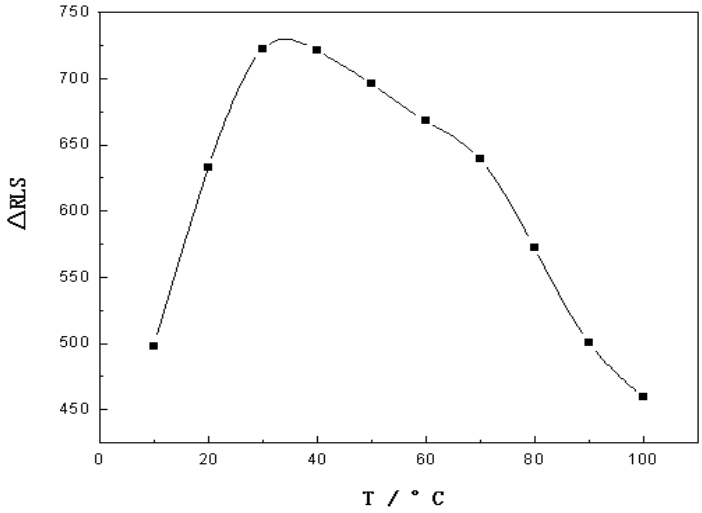

Fig. 6: Effect of temperature on the intensity of RLS (pH 8.1; c_{DNA} 5.0×10^{-6} g/L; $c_{o\text{-}HPF}$ 2.0×10^{-7} mol/L)

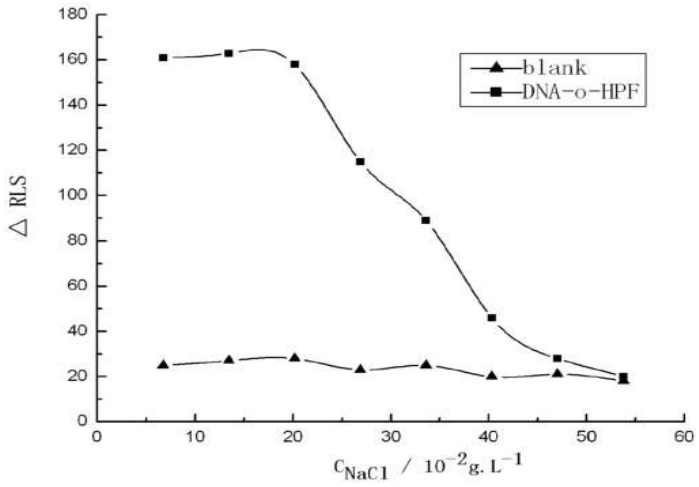

Fig. 7: Effect of ionic intensity on the intensity of RLS (pH 8.1; c_{DNA} 5.0×10^{-6} g/L; $c_{o\text{-}HPF}$ 2.0×10^{-7} mol/L)

Table 1: Interference of foreign substances

Foreign substances	The max. allowable concentration (mg/L)	RSD (%)	Foreign substances	The max. allowable concentration (mg/L)	RSD (%)
Na^+	134	-2.51	Ni^{2+}	2.50	5.01
K^+	216	-3.10	Li^+	0.20	2.33
Zn^{2+}	5	2.72	Pb^{2+}	1.6×10^{-5}	-2.30
Co^{2+}	0.20	-0.46	Cu^{2+}	0.05	-1.37
Dodecyl sodium sulfate	9.50	2.78	Glutamic acid	512	1.03
BSA	0.01	5.95	Lactamine	508	3.83

Max.: Maximum

Table 2: The results of determinations in synthesis samples

Concentration of DNA (10^{-6} g/L)	Foreign substances (10^{-6} g/L)	Found (10^{-6} g/L)	RSD (%)
2.50	Zn^{2+} (100) Co^{2+} (100)	2.47	5.3
3.50	K^+ (1000) Lactamine (2000)	3.51	3.9
4.50	Ni^{2+} (100) Glutamic acid (1000)	4.52	3.0

addition, the excess Na ions screened the positive charge on the DNA backbone, impaired the interaction between the negatively charged dye *o*-HPF with a positive charge ionic of DNA, which was not conducive to the arrangement of *o*-HPF on DNA, so that ΔRLS intensity decreased in the solution with high ionic strength. Therefore, the experiment should avoid the presence of high concentrations of electrolytes.

Tolerance of foreign substances: Under the optimum conditions, the effect of some foreign substances including Na^+, K^+, Zn^{2+}, Co^{2+}, Li^+, Ni^{2+}, Pb^{2+}, glutamic acid, lactamine, dodecyl sodium sulfate and BSA on the determination was investigated. The experimental results were listed in Table 1. It was found that most of the heavy metal ions could be tolerated at low concentration indicating that these metal ions had great influence on the determination of DNA. Therefore, these ions should be removed before measurement through some chemical methods, such as adding shielding agent, in order to eliminate the interference. Common biological sample Na and K ions had little effect, indicating their presence in a certain range of error would not be greatly affected. The coexistence of protein affected the determination of DNA, because the optimized measured wavelength of *o*-HFP-BSA system located at 523 nm, which was near to our working wavelength (Liu *et al*., 2008).

Calibration curve: The calibration curve was obtained according to the above standard procedure. There were linear relationship between the ΔRLS intensity and the concentration of DNA when the concentration of DNA was in the range of 0.02-1.8×10^{-5} g/L. The linear regression equation is:

$$I = -29.514 + 9.61023\ c\ (\times10^6),\ r = 0.9979$$

And the limit of determination of determination (3σ) was 4.6×10^{-8} g/L.

Analytical application in synthetic samples: To validate the applicability of this approach, the method was further applied to determine DNA in three synthetic samples containing different concentration of DNA. The experimental data were listed in Table 2. As is shown that, the determination results were reproducible and reliable.

CONCLUSION

A method for determination of DNA was developed based on the enhanced RLS technique. In this study, *o*-HFP synthesized in our own laboratory was used to determine DNA. In the Tris-HCl buffer solution, the addition of DNA enhanced the RLS signal of *o*-HFP and the enhanced intensity was direct proportion to DNA concentration in the range of 0.02-1.8×10^{-5} g/L with the detection limit of 4.6×10^{-8} g/L. The method was proved to be simple, rapid and high accuracy which had been applied to the determination of DNA in sample with satisfactory results.

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