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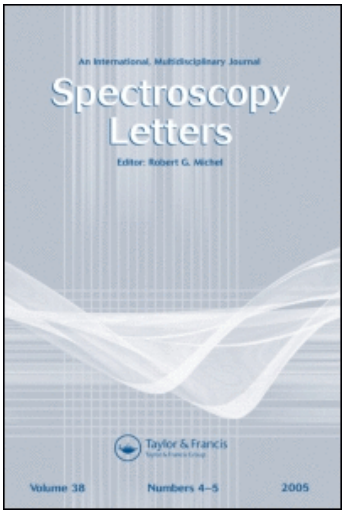


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# Determination of Nucleic Acids Sensitized by Emulsifier OP-micelle Using Ethyl Rhodamine B as a Resonance Light-Scattering Probe

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**Abstract:** The resonance light scattering (RLS) spectra of ethyl rhodamine B with nucleic acid (calf thymus DNA and herring sperm DNA) have been studied. The effective factors and the optimum conditions have been studied, and the enhanced intensity of RLS is in proportion to the concentration of nucleic acids in the range $0 \sim 5.00\ \mu g\ mL^{-1}$ for calf thymus DNA (ctDNA) and $0 \sim 3.50\ \mu g\ mL^{-1}$ for herring sperm DNA (hsDNA). The limits of detection are 3.42 and 3.14 ng $mL^{-1}$, respectively. Based on this, a RLS method for the determination of nucleic acids sensitized by emulsifier OP-micelle was accordingly established. The binding mode concerning the interactions of ethyl rhodamine B with nucleic acids was also studied, and this method has good selectivity and high sensitivity and it has been

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applied to the determination of DNA in synthetic samples and real samples with satisfactory results.

**Keywords:** Emulsifier, ethyl rhodamine B, nucleic acid, OP-micelle, resonance light scattering (RLS), spectral probe

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## INTRODUCTION

As an important genetic material, nucleic acid determination continues to be an active area of investigation in the fields of chemical and biochemical analysis. Quantitative determination of nucleic acids is a basic requirement of other studies. In 1993, by using a spectrofluorometer to measure resonance light scattering (RLS), Pasternack and co-workers studied the J aggregations of porphyrins,[1] which makes the RLS technique widely applied to the fields of biochemical analysis. Later on, Huang et al. discovered that by using the enhanced RLS intensity,[2] trace amounts of nucleic acids can be sensitively determined. When probes react with nucleic acids, based on the resonance energy transfer, their fluorescence intensity can be generated or enhanced. Using a spectrofluorometer, the molecular scattering spectra can be obtained conveniently,[3] the biomacromolecules whose molecules have been assembled or aggregated can be determined sensitively,[4,5] and the aggregation of molecule dyes in presence of surfactants or biomacromolecules can be characterized. [6] When the wavelength of the incident beam is close to that of the absorption band of the molecular particles, the intensity of resonance light scattering will rapidly increase.[7] The RLS intensity is in proportion to the concentration of the scattering particles in a certain range, which can be applied to determine the scattering particles quantitatively. The method established based on this is resonance light scattering technique.

Recently, many organic dyes have been used to determine nucleic acid using this method including rhodamine B[8] and butyl rhodamine B[9,10] instead of ethyl rhodamine B. To our knowledge, the use of ethyl rhodamine B for the determination of nucleic acid sensitized by emulsifier OP-micelle has not been reported. The novelty of this paper is that we used emulsifier OP-micelle to enhance the sensitivity of this system, and the binding mode concerning the interactions of ethyl rhodamine B with nucleic acids had been studied. What's more, it had been applied to the determination of DNA in real samples. The RLS spectra of ethyl rhodamine B with calf thymus DNA (ctDNA) in the acidic medium has been researched. The structure of ethyl rhodamine B is as follows in Fig. 1. The addition of DNA enhances the scattering intensity of the solution at 387 nm in OP-micelle. Based on this, a RLS method for the determination of nucleic acids at nanogram levels was accordingly established. In terms of the mechanism, there are three binding modes concerning the interactions of dyes with nucleic acids: intercalative binding, groove binding, and electrostatic

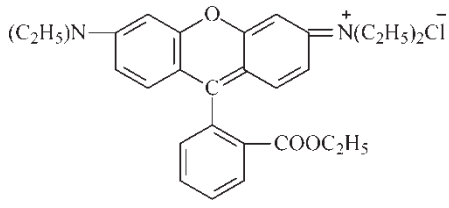


*Figure 1.* The structure of ethyl rhodamine B.

binding. Then the binding mode concerning the interactions of ethyl rhodamine B with nucleic acids was also studied. This method is simple, rapid, and is also superior to most of the reported probe methods with respect to its sensitivity (Table 1), and it has been applied to the determination of DNA in the synthetic samples and real samples with satisfactory results.

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## MATERIALS AND METHODS

### Apparatus

Intensity and spectra of RLS were obtained with a Perkin-Elmer Model LS-55 Luminescence Spectrometer (Perkin-Elmer, NY, USA) using 1.0-cm quartz cells. Absorbance measurements and absorption spectral records were obtained with a Model UV-3101PC spectrophotometer (Shimadzu, Japan). A Model PHS-3B pH meter (Leici, Shanghai, China) was employed for pH measurements.

### Reagents

Stock solutions of nucleic acids were prepared by directly dissolving commercial ctDNA (Beijing Jingke Company, Beijing, China), herring sperm DNA (hsDNA; Sigma, USA) in doubly deionized water at $0 \sim 4^{\circ}C$ under occasional gentle shaking. Twenty-four hours or more were needed for dissolving DNAs. The exact concentrations of nucleic acids were determined by measuring the absorbances at 260 nm. The standard solutions of each nucleic acid were prepared with 50.0 $\mu g\ mL^{-1}$, and the working solutions were made by dilution.

Ethyl rhodamine B solution ($3.0 \times 10^{-4}\ mol\ L^{-1}$) was prepared by dissolving 0.0076 g of ethyl rhodamine B in ethanol, which was then brought to 50 mL in a volumetric flask. The working solution was obtained by diluting the stock solution with ethanol to the required concentration.

OP-micelle (5%) was prepared by dissolving 5.0 g of emulsifier OP (OP) and diluting with water to 100 mL in a volumetric flask.

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***Table 1.*** Some resonance Rayleigh scattering methods for the determination of nucleic acid

| Reagent | Nucleic acid | pH | $\lambda_{RLS}$(nm) | Linear range (μg $mL^{-1}$) | Detection limit (ng $mL^{-1}$) | Reference |
|---|---|---|---|---|---|---|
| Rhodamine B | ctDNA | 1.00 | 378.0 | 0.1∼16.0 | | [8] |
| Butyl rhodamine B | DNA | 1.5∼2.5 | 400 | 0.0058∼2.688 | 5.8 | [9] |
| Butyl rhodamine B | DNA | 1.5∼2.5 | 400 | 0.0058∼2.688 | 5.8 | [10] |
| Dequalinium chloride | ctDNA | 7.00 | 362.0 | 0.04∼10.0 | 6.2 | [11] |
| CTMAB | ctDNA | | 414 | 0.025∼20 | 8.3 | [12] |
| CPB | ctDNA | 9.50 | 350.0 | 0.005∼50.0 | 4.3 | [13] |
| Manganese-tetrasulfonateophthalocyanine | ctDNA | 11.6 | 346.2 | 0.4∼1.4 | 22.9 | [14] |
| CuTSPc | fsDNA | 10.5 | 383.6 | 0.4∼1.5 | 10.6 | [15] |
| CuTSPc | ctDNA | 10.5 | 383.6 | 0.4∼1.0 | 32.4 | [15] |
| Acetamide | DNA | 1.6∼1.8 | 313 | 0∼11 | 20 | [16] |
| MnTSPc | ctDNA | 11.6 | 346.2 | 0.4∼1.4 | 22.9 | [17] |
| Acridine yellow | fsDNA | 5.5 | 465 | | 10.69 | [18] |
| $[Fe(phen)(dpy)]^{2+}$ | ctDNA | 9.7∼9.9 | 418 | 0.04∼0.35 | 36 | [19] |

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| | | | | | | |
|---|---|---|---|---|---|---|
| Alkali blue 6B | fsDNA | 2.9 | 346 | 0.01 ~ 0.1004 | 4.2 | [20] |
| La(phen)($C_8H_6O_4$) | ctDNA | 6.5 ~ 7.5 | 400 | 0.023 ~ 1.87 | 23 | [21] |
| Thionine | ctDNA | 2.2 | 340 | 0 ~ 10 | 3.5 | [22] |
| Poly(ethyleneimine) | hsDNA | | 300 | 0.01 ~ 10 | 5.3 | [23] |
| $TC_{16}PyP$ | ctDNA | 10.24 | 311.8 | 0.2 ~ 6 | 16 | [24] |
| ZnS | ctDNA | 9.5 | 304.5 | 0.04 ~ 1.2 | 19 | [25] |
| Pyronine B | ctDNA | 7.4 | 328 | 0 ~ 1.2 | 6.1 | [26] |
| Safranine T | ctDNA | 7.05 | 328.0 | 0 ~ 2.5 | 13.2 | [27] |
| Brilliant cresol blue | ctDNA | 7.40 | 364.0 | 0.118 ~ 4.70 | 118.0 | [28] |
| Ethyl rhodamine B | ctDNA | 1.30 | 387 | 0 ~ 5.00 | 3.42 | This paper |

CTMAB: cetyltrimethyl ammonium bromide.
CPB: cetylpyridinium bromide.
CuTSPc: Copper Phthalocyanine Tetrasulfonic Acid.
fsDNA: fish sperm DNA.
MnTSPc: Manganese-tetrasulfonato phthalocyanine.
$TC_{16}PyP$: Tetra-(*N*-hexadecyl pyridiniumyl) Porphyrin.

Hydrochloric acid solution (1.0 mol $L^{-1}$) was obtained by diluting hydrochloric acid with water.

All the chemicals used were of analytical reagent grade, and doubly deionized water was used throughout.

## Procedure

To a 10 mL test tube, solutions were added in the following order: 0.5 mL of 1.0 mol $L^{-1}$ HCl solution, 1.0 mL of 5% OP-micelle, 0.16 mL of $3.0 \times 10^{-6}$ mol $L^{-1}$ ethyl rhodamine B solution, and definite standard nucleic acids or sample solution. The solution in the test tube should be homogenized by shaking after each addition of reagents. Finally, the mixtures were diluted to 10 mL with water and stood for 10 min. The RLS spectra were measured by scanning synchronously at the same excitation and emission wavelengths. The intensity of RLS against the blank was obtained at 387 nm with slit width at 15 nm for the excitation and 3.3 nm for the emission.

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## RESULTS AND DISCUSSION

### Absorption Spectra

From RLS spectra (Fig. 2), it can be seen that the addition of DNA enhances the scattering intensity of ethyl rhodamine B solution at 387 nm, 462 nm, and 484 nm, and these enhanced RLS signals increase with increasing concentration of nucleic acids. According to the theory of RLS,[29] the RLS

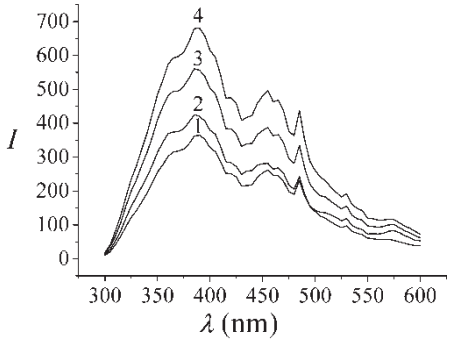


***Figure 2.*** RLS spectra of ethyl rhodamine B–ctDNA system. Conditions: (1) ethyl rhodamine B; (2) ethyl rhodamine B–DNA (0.5 μg $mL^{-1}$); (3) ethyl rhodamine B–DNA(1.0 μg $mL^{-1}$); (4) ethyl rhodamine B–DNA (1.5 μg $mL^{-1}$); $c_{\text{ethylrhodamineB}} = 4.8 \times 10^{-8}$ mol $L^{-1}$. $V_{OP} = 1.0$ mL; pH = 1.30.

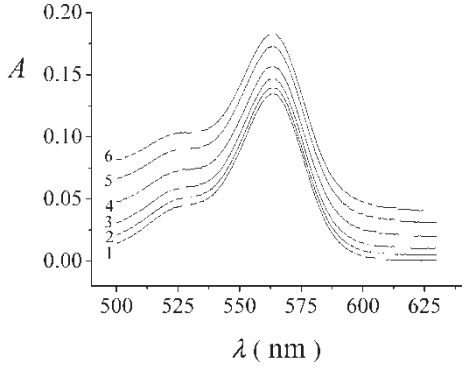


***Figure 3.*** Conditions: (1) ethyl rhodamine B; (2) ethyl rhodamine B–DNA (2.5 μg $mL^{-1}$); (3) ethyl rhodamine B–DNA (5.0 μg $mL^{-1}$); (4) ethyl rhodamine B–DNA (7.5 μg $mL^{-1}$); (5) ethyl rhodamine B–DNA (10.0 μg $mL^{-1}$); (6) ethyl rhodamine B–DNA (12.5 μg $mL^{-1}$); $c_{\text{ethylrhodamineB}} = 1.2 \times 10^{-6}$ mol $L^{-1}$. $V_{OP} = 1.0$ mL; pH = 1.30.

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spectra had peaks situated at minimum absorption. Thus in this paper, the 387-nm peak was chosen for further study.

Absorption spectra of ethyl rhodamine B–ctDNA system are shown in Fig. 3. It can be seen from Fig. 3 that ethyl rhodamine B has an absorption peak at 564 nm at pH 1.30. The interaction of ctDNA with ethyl rhodamine B results in a great increase in the absorption peak of ethyl rhodamine B at 564 nm. The increased absorption signals enhance with increasing concentration of nucleic acids. In terms of the mechanism, there are three binding modes concerning the interactions of dyes with nucleic acids: intercalative binding, groove binding, and electrostatic binding. When a spectral embedded probe embeds in the base pairs of nucleic acid, it can cause red shift, isoabsorptive point, and hypochromic effect of the spectrum. From Fig. 3, we can see that the addition of ctDNA can cause the hyperchromic effect of the system's spectrum. These characteristics are consistent with the change of the spectrum caused by groove binding. Thus it can be concluded that the binding mode concerning the interactions of ethyl rhodamine B with nucleic acids is groove binding.

## Effect of Acidity

The RLS intensity of the system was significantly affected by acidity. It was found that the RLS intensity of ethyl rhodamine B was enhanced by ctDNA in acidic medium (Fig. 4). It reached the maximum value and remained constant in the pH range $1.0 \sim 1.5$. Thus the optimum pH to run the assay was pH 1.30, and 1.5 mL of 0.1 mol $L^{-1}$ hydrochloric acid solution was used to adjust the pH.

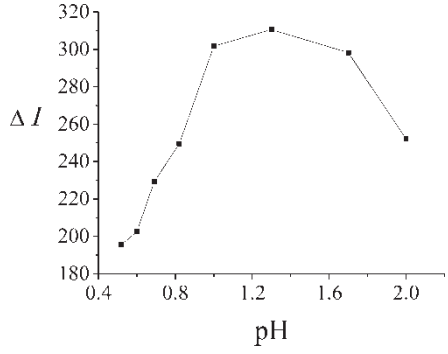


***Figure 4.*** Influence of pH. $c_{\text{ethylrhodamineB}} = 4.8 \times 10^{-8}$ mol L$^{-1}$; $c_{\text{ctDNA}} =$ 1.5 μg mL$^{-1}$; $V_{\text{OP}} = 1.0$ mL.

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## Effect of Different Media

In the experiment, aqueous medium, OP, Tween 20, Tween 80, Triton X-100 micellar solution, and β-cyclodextrin were chosen for investigating the effect of medium on the intensity, as shown in Fig. 5. Different kind of medium results in different effects on the scattering intensity of the system. The effect of OP-micellar solution was the most remarkable, and it enhanced the intensity greatly. Its suitable addition amount was 1.0 mL. Thus we used OP-micellar solution as the medium of this system.

## Effect of Ethyl Rhodamine B Concentration

The influence of concentration of ethyl rhodamine B was also tested. From Fig. 6, it could be seen that with the increase of ethyl rhodamine B

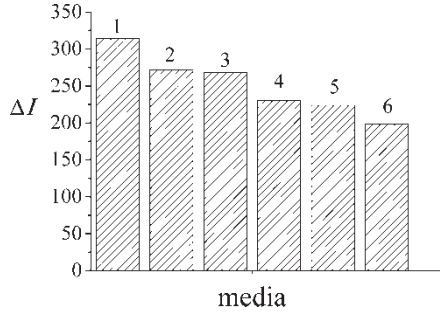


***Figure 5.*** Effect of different media on the intensity. (1) OP; (2) Triton X-100; (3) Tween 20; (4) Tween 80; (5) β-cyclodextrin; (6) aqueous medium; Conditions: $c_{\text{ethylrhodamineB}} = 4.8 \times 10^{-8}$ mol L$^{-1}$; $c_{\text{ctDNA}} = 1.5$ μg mL$^{-1}$; pH = 1.30.

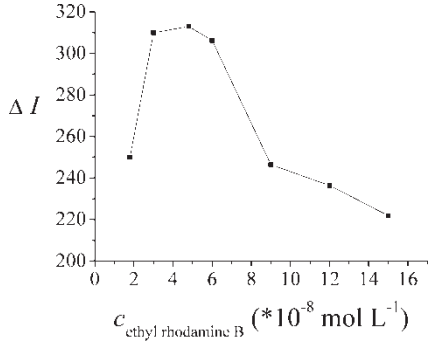


***Figure 6.*** Effect of the amount of ethyl rhodamine B. Conditions: $c_{\text{ctDNA}} = 1.5\ \mu\text{g mL}^{-1}$; pH = 1.30; $V_{\text{OP}} = 1.0$ mL.

concentration, the RLS intensity enhanced gradually and kept stable in the concentration range $3.0 \times 10^{-8}$ to about $6.0 \times 10^{-8}$ mol L$^{-1}$, but if ethyl rhodamine B concentration was too high, on the other hand, the RLS intensity decreased. Therefore, $4.8 \times 10^{-8}$ mol L$^{-1}$ of ethyl rhodamine B was chosen as the optimal concentration for the system.

## Reaction Time and Stability

Tests showed that the RLS intensity reached a maximum 10 min after all the reagents had been added and remained stable for about 10 hr. In this study, 10 min was set as the standard for measurements.

## Effect of Ionic Strength

The effect of ionic strength on the light scattering intensity of ctDNA was studied, in which 0.1 mol L$^{-1}$ NaCl was used to control the ionic strength. As can be seen from Fig. 7 the $\Delta I_{\text{RLS}}$ kept constant when the NaCl concentration was less than 0.01 mol L$^{-1}$. The experiments also showed that the $\Delta I_{\text{RLS}}$ was immediately decreased as soon as more NaCl solutions were added. It can be explained as follows: in the high-concentration NaCl solution, when ctDNA reacts with ethyl rhodamine B, $Na^+$, $Cl^-$ can cause competition and shielding action to the system, which makes the $\Delta I_{\text{RLS}}$ decrease.

## Effect of Addition Order of the Reagents

The addition order of the reagents had a substantial effect on the enhanced RLS intensity. In this work, the addition order of the reagents was selected as follows: buffer solution, OP micellar solution, ethyl rhodamine B, nucleic acid.

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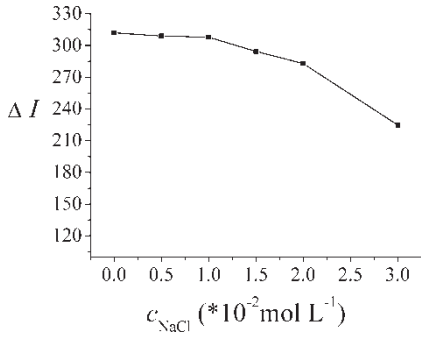


***Figure 7.*** Effect of ionic strength. Conditions: $c_{\text{ethylrhodamineB}} = 4.8 \times 10^{-8}$ mol $L^{-1}$; $c_{\text{ctDNA}} = 1.5$ μg $mL^{-1}$; pH = 1.30; $V_{OP} = 1.0$ mL.

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## Effect of Denaturation of Nucleic Acids

Some ctDNA solution was added to a test tube and was heated for 15 min in a boiling water bath and then cooled for 5 min in ice water to give denatured ctDNA. A parallel test with natural ctDNA and denatured ctDNA was carried out according to the recommended procedure. It can be seen that the resonance light scattering enhancement in the ethyl rhodamine B–denatured ctDNA system is weaker than that in the ethyl rhodamine B–natural ctDNA system.

## Effect of Foreign Substances

The interference of various ions, proteins, pyrimidines, and purines was tested according to the standard procedure (Table 2). It was found that these foreign

***Table 2.*** Tolerance of foreign substances (3.0 μg $mL^{-1}$ ctDNA)

| Foreign substances | Concentration coexisting (μg $mL^{-1}$) | Δ*I*(%) | Foreign substances | Concentration coexing (μg $mL^{-1}$) | Δ*I*(%) |
|---|---|---|---|---|---|
| $K^+$ | 10.8 | −3.58 | Thymine | 40 | 3.74 |
| $Al^{3+}$ | 0.1 | −2.58 | Uracil | 40 | −4.88 |
| $Ba^{2+}$ | 9 | −3.79 | Guanine | 30 | 3.54 |
| $Pb^{2+}$ | 0.62 | −4.81 | L-Lysine | 20 | 3.37 |
| $Ca^{2+}$ | 0.004 | −4.85 | L-Tyrosine | 40 | 2.91 |
| $Mn^{2+}$ | 0.8 | −3.78 | DL-Valine | 20 | 2.68 |
| $Cu^{2+}$ | 0.004 | −3.79 | L-Arginine | 22 | 4.17 |
| $Co^{2+}$ | 0.002 | 4.04 | L-Histidine | 24 | 4.20 |
| $Cd^{2+}$ | 0.337 | 3.70 | L-Tryptophan | 22 | 3.20 |
| $Ni^{2+}$ | 0.352 | 2.67 | Glycolamine | 20 | 2.45 |

substances had little effect on the determination of nucleic acids under the permission of 5% error.

## Calibration Graphs and Sensitivity of the Method

Under the conditions given above, a linear relationship was obtained between the $\Delta I_{RLS}$ and the concentration of DNA such as ctDNA and hsDNA. All the analytical parameters are summarized in Table 3. It can be seen that this method has low detection limit and high sensitivity.

## Analysis of Synthetic Samples and Real Samples

According to the interference of foreign substances, four synthetic samples were made, and the assay results are presented in Table 4. As can be seen from Table 4, the relative standard deviations are in all instances less than 4.0% and the recovery of nucleic acid is 94.0% to about 101.0%. All these present sufficient precision and high accuracy.

We have extracted DNA from cauliflower.[30] The process is as follows: Weigh out 30 g of cauliflower that has been frozen for more than 24 hr and cut it up. Infuse 10 mL of lapping liquid after putting the broken cauliflower into the mortar and grind for more than 10 min. Centrifuge the mixture and pour the pellucid liquid into the double-volume of 95% cold alcohol and mix a round slowly and lightly with a glass stick. After 35 min of sedimentation, the floccule of DNA appears. Revolving the glass stick, the floccule will entwine it.

Then, concentration of the DNA solution extracted from cauliflower was determined by the proposed method, and in order to prove the possibility of using this method for analysis of real samples, the concentration of DNA extracted from cauliflower was first detected by classic method using ethidium bromide and the result was 17.3 $\mu g\ mL^{-1}$. The result achieved by standard addition method was 17.9 $\mu g\ mL^{-1}$, close to the data provided by the classic method, and the relative error was 3.47% (Table 5). The recovery of DNA was also tested, and the analytical results was 97.1% to about 102.5%, which was satisfactory (Table 6). It

***Table 3.*** Analytical parameters for nucleic acids

| Nucleic acid | Linear range ($\mu g\ mL^{-1}$) | Linear regression equation ($\mu g\ mL^{-1}$) | $r$ | Detection limit ($ng\ mL^{-1}$) |
|---|---|---|---|---|
| ctDNA | $0 \sim 5.0$ | $\Delta I_{RLS} = 13.73 + 190.8\rho$ | 0.9956 | 3.42 |
| hsDNA | $0 \sim 3.5$ | $\Delta I_{RLS} = -7.00 + 215.5\rho$ | 0.9999 | 3.14 |

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***Table 4.*** The results for the determination of synthetic samples[a]

| Nucleic acid in the samples | ($\mu g\ mL^{-1}$) | Main interference | Samples found[b] ($\mu g\ mL^{-1}$, $n = 5$) | [c]RSD (%) | Recovery (%, $n = 5$) |
|---|---|---|---|---|---|
| ctDNA | 15.00 | Thymine, Guanine, $K^+$, $Pb^{2+}$ | $13.72 \pm 0.18$ | 3.9 | 94.8 |
| ctDNA | 15.00 | L-Tyrosine, L-histidine, $Ba^{2+}$, $Mn^{2+}$ | $14.11 \pm 0.15$ | 2.3 | 94.1 |
| hsDNA | 15.00 | Thymine, guanine, $K^+$, $Pb^{2+}$ | $15.21 \pm 0.19$ | 1.9 | 101 |
| hsDNA | 15.00 | L-tyrosine, L-histidine, $Ba^{2+}$, $Mn^{2+}$ | $14.54 \pm 0.21$ | 1.9 | 96.9 |

[a]Conditions: thymine, 4.0 $\mu g\ mL^{-1}$; guanine, 3 $\mu g\ mL^{-1}$; $K^+$, 2.5 $\mu g\ mL^{-1}$; $Pb^{2+}$, 0.50 $\mu g\ mL^{-1}$; L-tyrosine, 4.0 $\mu g\ mL^{-1}$; L-histidine, 2.0 $\mu g\ mL^{-1}$; $Ba^{2+}$, 2.0 $\mu g\ mL^{-1}$; $Mn^{2+}$, 0.50 $\mu g\ mL^{-1}$.

[b]Results expressed as: $\bar{x} \pm st/\sqrt{n}(n = 5)$ where $\bar{x}$ is the mean of $n$ observations of $x$, $s$ is the standard deviation, and $t$ is distribution value chosen for the desired confidence level. Theoretical values at 95% confidence limit: $t = 2.78$.

[c]RSD: Relative standard deviation.

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clearly indicates that the current method is accurate, sensitive, reliable, precise, and simple.

## CONCLUSIONS

The light scattering technique is useful in developing a quantitative method in chemical and biochemical fields. Because all the data are obtained by using a spectrofluorometer, the main advantages of the technique are its high sensitivity and simplicity in handling. In this paper, the nature of RLS of ethyl rhodamine B–nucleic acid system and the binding mode concerning the interactions of ethyl rhodamine B with nucleic acids was first studied. The

***Table 5.*** The test of DNA sample extracted from cauliflower

| Sample | Standard addition method ($\mu g\ mL^{-1}$) | Classic method ($\mu g\ mL^{-1}$) | Relative error (%) |
|---|---|---|---|
| 1 | 17.6 | 17.1 | 2.92 |
| 2 | 18.1 | 17.6 | 2.84 |
| 3 | 18.0 | 17.2 | 4.65 |
| Mean found | 17.9 | 17.3 | 3.47 |

***Table 6.*** The recovery test of DNA extracted from cauliflower

| DNA added ($\mu g\ mL^{-1}$) | DNA found ($n = 5$) ($\mu g\ mL^{-1}$) | Recovery (%) | [a]RSD (%) |
|---|---|---|---|
| — | 10.00 | — | 2.5 |
| 20.00 | 30.75 | 102.5 | 2.4 |
| 30.00 | 38.84 | 97.1 | 3.5 |

[a]RSD: Relative standard deviation.

relationship between the $\Delta I_{RLS}$ and the concentration of nucleic acid was established. This method has some advantages such as simplicity of operation, stability of complex formed, and high sensitivity. It has been applied to the determination of DNA in the synthetic samples and real samples with satisfactory results.

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