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Resonance Rayleigh scattering method for the recognition and determination of double-stranded DNA using amikacin

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Abstract

In weakly acidic buffer medium, the interaction of amikacin with calf thymus DNA, yeast RNA and denatured DNA has been investigated by using resonance Rayleigh scattering (RRS) technique. The result shows that calf thymus DNA is capable of enhancing the RRS intensity of the amikacin, while yeast RNA and denatured DNA have very little enhancement effect. Based on the characteristics, a sensitive assay for detecting double-stranded DNA in the presence of denatured DNA and yeast RNA has been developed. The enhancement of the RRS signal is directly proportional to the concentration of double-stranded DNA in the range 0.02–12.0 $\mu g\,ml^{-1}$ for calf thymus DNA and its detection limit (3σ) is 2.5 $ng\,ml^{-1}$. The method shows a wide linear range and high sensitivity, and almost no interference can be observed from RNA, denatured DNA, amino acid and most of the metal ions. The trace amounts of nucleic acid in synthetic samples and practical samples are determined with satisfactory results. Therefore, the proposed method is promising for as an effect means for recognition in vivo and determination in situ of double-stranded DNA.

Keywords: Resonance Rayleigh scattering (RRS); Double-stranded DNA; Amikacin

1. Introdution

Nucleic acid is a sort of important biological macromalecule, and the quantification of nucleic acids, in particular double-stranded (ds) DNA, is crucial in many molecular biological and diagnostic applications. These include measurements of nucleic acids in biological material, determinations of cell concentration or cell proliferation, as well as the pre-, online-, or postevaluation of polymerase chain reactions (PCR) [1]. Generally, the conventional spectrophotometric method used for nucleic acids assay is based on the measurement of ultraviolet absorption at 260 nm. However, by this method only pure nucleic acid preparations can be quantified correctly [2]. Some organic reagents, which can react with nucleic acids, have been developed for spectrophotometric methods of nucleic acids. These determinations are precise and reliable, but they often suffer from the disadvantage of low sensitivity or procedural complications [3–5]. The fluorometric methods are based on the quench or enhancement of the fluorescence of various compound (e.g. ethidium bromide (EB) and its dimmer, Hoechest33258, thiazide orange homodimer (TOTO) and oxazide yellow homodimer (YOYO), as well as Tb(III), Eu(III) and their chelates [6–12], etc.) after they interact with nucleic acids. The methods have high sensitivity, but they are only limited to the fluorescent systems and some fluorescent dyes are expensive and are strong carcinogenic reagents.

Resonance Rayleigh scattering (RRS) is a special elastic scattering produced when the wavelength of Rayleigh scattering (RS) is located at or close to its molecular absorption band. In this case, the frequency of the electromagnetic wave absorbed by the electron is equal to its scattering frequency. Owing to the intensive absorption of light energy of the electron, re-scattering takes place. Therefore, the scattering intensity is enhanced several orders of magnitude compared with single RS and no longer obeys the Rayleigh law of $I \propto \lambda^{-4}$ [13]. RRS shows also the characteristics of the scattering spectrum as well as that of the electronic absorption spectrum. Compared with a single RS technique, it not only has high sensitivity and better selectivity, but also can provide a plenty of new information concerning molecular structure, size, form, charge, distribution, state of

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combination, and so on. In recent years, this technique has been increasingly applied to the study and determination of some biological macromolecules, as well as trace amounts of inorganic ions, and cationic surfactant [14–18]. The determination of nucleic acids is mainly based on the fact that the aggregation of chromophores on nucleic acids can give rise to strong RRS [19]. At present, the reagents used as the chromophores are cationic dyes, (e.g., methylene blue, brilliant cresol blue, and thionine, etc.) [14,15,20–25], as well as the cationic chelate of Co(II)-5-Cl-PADAB [26]. In addition, some proteins and drugs (e.g. histone, dequalinium chloride, etc.) [27,28] have been also used to determinate nucleic acids by RRS methods. The above RRS methods are available for quantification of nucleic acids in solution, but none has been described that is both sensitive and specific for dsDNA. Here we show, for the first time, that amikacin can be successfully employed as a probe for recognition and sensitive mesurement of dsDNA in solution by using RRS method.

Amikacin (AK) is a semisynthetic, water soluble, broad spectrum aminoglycoside antibiotic. It is commonly administered parenteral for the treatment of Gram-negative infections resistant to gentamicin, kanamycin or tobramycin because the AK molecule has fewer points susceptible to enzymatic attack than most other aminoglycosides. However, it has been well known that they could cause damage to the kidneys and cranial nerves [29,30]. Therefore, it is important to study the reaction of AK with nucleic aicd. Our experiment shows that in weakly acidic medium, AK has very weak RRS. However, when it coexists with dsDNA, they can combine to form supermolecule compound, in which AK molecules assemble on the DNA. This can lead to a great enhancement of RRS intensity and appearance of new RRS spectra. In contrast, the reaction between AK and single-strand (ss) DNA or yeast RNA (yRNA) does not produce the great enhancement of RRS intensity. Based on the characteristics, a sensitive assay for detecting dsDNA in the presence of denatured DNA and yRNA has been developed. The enhancement of the RRS signal is directly proportional to the concentration of DNA in the range 0.02–12.0 $\mu g\,ml^{-1}$ for calf thymus DNA (ctDNA), and its detection limit (3σ) is 2.5 $ng\,ml^{-1}$. 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 recognition and determination of dsDNA, for the first time, is established.

2. Experimental

2.1. Reagents and apparatus

A Shimadzu RF-850 spectrofluorometer (Kyoto, Japan) was used for measuring the RRS intensity at a given wavelength using a 1 cm path length. The determined parameters were low sensitivity and the slit (EX/EM) of 10.0 nm/10.0 nm.

The stock solutions of DNA and RNA were prepared by dissolving commercially purchased ctDNA and yRNA, (All nucleic acids were all purchased from Hua Mei Institute of Biochemistry, China) in water. For DNA, ≥24 h were needed at 4 °C with gentle shaking occasionally. The concentrations of nucleic acids were determined according to the absorbances at 260 nm after establishing that the absorbance ratio A_{260}/A_{280} was in the range 1.80–1.90 for ctDNA and 1.90–2.00 for yRNA. All solutions of the nucleic acids were kept in a refrigerator at 4 °C. 0.1 $mol\,l^{-1}$ NaAc–HAc buffer solution was used to control the pH value of the interacting system, while 1.0 $mol\,l^{-1}$ NaCl was used to adjust the ionic strength of the aqueous solution.

The denaturation of DNA was achieved by heating dsDNA solution in water bath at about 100 °C for 100 min, followed by rapid cooling in ice bath.

The stock solution of Sulfate Amikacin (Shandong Lukang Medicine Plant) was 400.0 $\mu g\,ml^{-1}$, and the working concentration was 60.0 $\mu g\,ml^{-1}$ by diluting the stock concentration with water.

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

2.2. Procedures

Into a 10 ml calibrated flask are added 2.0 ml NaAc–HAc buffer solution, 1.0 ml 60.0 $\mu g\,ml^{-1}$ AK solution and appropriate nucleic acid solution, and then dilute it to 10 ml with doubly distilled water. After mixing thoroughly the solution, record the RRS spectra with synchronous scanning at $\lambda_{em} = \lambda_{ex}$ and measure the RRS intensity I_{RRS} for the reaction product and I_0 for the reagent blank at the maximum scattered wavelength, $\Delta I = I_{RRS} - I_0$.

2.3. Determination of DNA in synthetic samples by RRS method

Place 2.0 ml NaAc–HAc buffer solution and 1.0 ml, 60.0 $\mu g\,ml^{-1}$ AK solution into a 10 ml calibrated flask, then add 1.0 ml synthetic samples and mix thoroughly. The following procedure is the same as above.

2.4. Determination of plasmid DNA by UV-Vis spectrocopy and RRS method

The concentration of plasmid DNA extraction from *E. coli*, obtained from China Pharmaceutical University, is determined from the absorbance at 260 nm, assuming an absorbance of 1.0 for 50 μg DNA $(ml\,cm)^{-1}$. Then place 1.0 ml plasmid DNA samples into a 10 ml calibrated flask, the following procedure is the same as above. The differences of two methods were compared.

3. Results and discussions

3.1. Spectral characteristics and reaction

Fig. 1 shows the RRS spectra of the AK, ctDNA and AK-ctDNA complex from 250 to 800 nm. It can be seen that AK itself has weak RRS intensity. Under the same conditions, the RRS intensity of DNA is faint even if its concentration reached 10.0 μg ml^{-1}. However, when DNA and AK are mixed together, the intensity of RRS is enhanced in the range of 300–500 nm. The maximum scatter peak is at 300 nm, and there are four RRS peaks at 350, 410, 470 and 560 nm, respectively. It can be concluded that the interaction between AK and ctDNA has occurred. These enhanced RRS signals increase with increasing concentration of ctDNA (see Fig. 1). But when the concentration of ctDNA is higher than 12.0 μg ml^{-1}, the RRS intensity of the system will not enhance. In this paper, the 300 nm peak is chosen for further study.

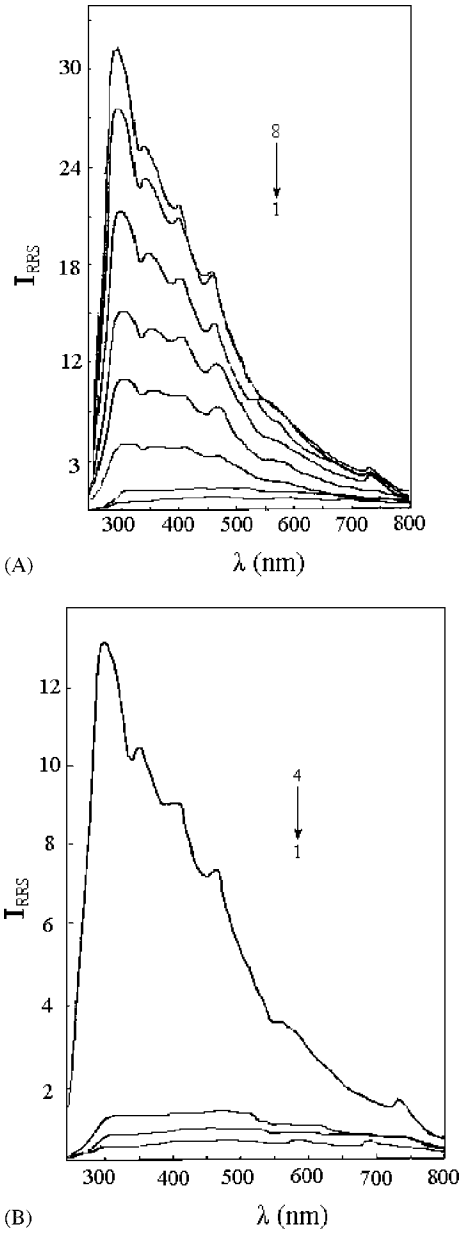

Fig. 1. Resonance Rayleigh scattering spectra of AK-nucleic acids system. A: AK-ctDNA system. (1) AK, 6.0 μg ml^{-1}; (2) ctDNA, 10.0 μg ml^{-1}; (3–8) AK-ctDNA complex. Concentration of ctDNA: 3, 1.0; 4, 2.0; 5, 3.0; 6, 5.0; 7, 7.0; 8, 10.0 μg ml^{-1}; AK, 6.0 μg ml^{-1}. B: AK-nucleic acids system. (1), AK; (2) AK-denatured DNA system; (3) AK-yRNA system; (4) AK-ctDNA complex. Concentration of AK, 6.0 μg ml^{-1}; denatured DNA, 6.0 μg ml^{-1}; yRNA, 6.0 μg ml^{-1}; ctDNA, 6.0 μg ml^{-1}.

In addition, we investigate the reactions of the thermal denaturation DNA and yRNA with AK. The phenomenon of the enhanced RRS intensity does not produce. Since yRNA is single stranded nucleic acid and thermal denaturation of double stranded nucleic acids produces single stranded nucleic acid, therefore, it is reasonable to infer that the interactions of AK with nucleic acids depend on the double stranded structure of nucleic acid. This phenomenon offers us an opportunity for recognition and determination dsDNA in the presence of ssDNA and RNA.

3.2. Optimum conditions for the reactions

3.2.1. Effects of solution acidity

The solution acidity has great affect on the scattering intensity of AK, DNA and their complex form (see Fig. 2). It can be seen from the Fig. 2 that the optimum pH range is 4.4–5.6. While pH value is lower than the range, an indirect proton-phosphate interaction via water and the protonation of nitrogen atoms of bases could occur [21]. Owing to the protonation of phosphate, both DNA and AK–DNA complex have strong RRS signals, and the RRS data are unstable. On the other hand, while the pH is higher than 5.6, there are probably less amino groups protonated in the AK molecule, which therefore lessens the electrostatic interaction between AK and DNA [30]. In order to obtain stable and high RRS data in this study, we should control

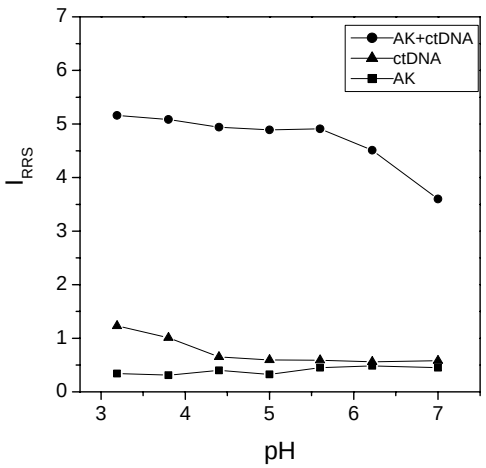

Fig. 2. Effect of solution acidity on the intensity of RRS. Concentration of AK: 6.0 μg ml^{-1}; ctDNA: 2.0 μg ml^{-1}.

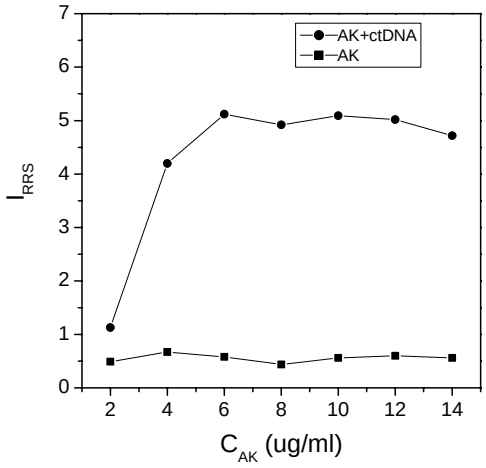

Fig. 3. Effect of AK concentration on the intensity of RRS. Concentration of ctDNA: 2.0 μg ml^{-1}.

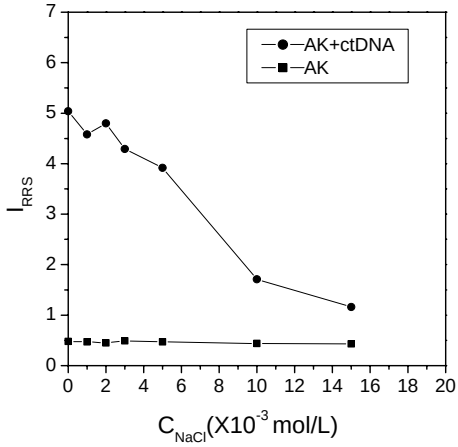

Fig. 4. Effect of ionic strength on the intensity of RRS. Concentration of AK: 6.0 μg ml^{-1}; ctDNA: 2.0 μg ml^{-1}.

the acidity of the interaction system at pH 5.6 with a 0.1 mol/l NaAc–HAc buffer solution. In addition, we investigated the influence of different buffer type, such as PBS, BR and NaAc–HAc buffer solution. The result shows that NaAc–HAc buffer solution is best.

3.2.2. *Effect of AK*

Fig. 3 is the effect of AK concentration. From the Fig. 3, it can be seen that the RRS intensity of the system is enhanced with the increase of the AK concentration. When the concentration of AK reaches 6.0 μg ml^{-1}, the RRS intensity reaches to the maximum values. A continuous increase in the concentration of AK hardly changes the RRS intensity. We select 6.0 μg ml^{-1} of AK as optimum concentration for determination of nucleic acids.

3.2.3. *The sequence of the reagents and the stability of the system*

The sequence of addition of the reagents affects the intensity of RRS. We investigate the effect of addition order of the reagents (see Table 1). The result show that mixing buffer and AK solution first and then adding nucleic acid solution can give a higher RRS intensity than other sequences of addition of the reagents. Under the optimal conditions of the reaction, the formation time of all reaction products is 5 min and the RRS intensity will keep constant in 1.5 h (in the range, the change of RRS intensity $\leq \pm 5\%$).

Table 1
Effect of addition order of the reagents

The order of the reagents	ΔI_{RRS} (%)	The order of the reagents	ΔI_{RRS} (%)
Buffer + AK + nucleic acid	100	AK + buffer + nucleic acid	88.6
Buffer + nucleic acid + AK	94.2	AK + nucleic acid + buffer	72.1
Nucleic acid + buffer + AK	98.5	Nucleic acid + AK + buffer	70.2

Under the same condition, we investigate the other drugs with positive charge (e.g. streptomycin sulfate, amitriptyline hydrochloride, chlorpromazine hydrochloride, doxepin hydrochloride) instead of AK to react with ctDNA. However, the phenomena of the enhanced RRS intensity of the systems are not observed. Therefore, we can conclude that AK as a probe can cognize selectively ctDNA by means of RRS method.

3.2.4. *The effect of ionic strength*

The effect of ionic strength on the RRS intensity is investigated with 1.0 mol/l NaCl. Ionic strength has obvious effect on the RRS intensity (see Fig. 4). Since in high ionic strength, the electrostatic force between Na^+ and the negative charged groups on nucleic acids is strengthened, which is unfavourable for the combination of AK with nucleic acids, the reaction is blocked. On the other hand, the effect of ionic strength on the reaction might result in the conformation variation of nucleic acids. As the concentration of NaCl increases, the flexible nucleic acid molecules become rigid, which inhibits the external (minor) groove binding or the interaction of AK [26]. Therefore the RRS intensity of the system is decreased, and the decrease of the RRS signal is nearly proportional to the ionic strength ($I = 5.08 - 0.28c$, $R = 0.979$).

3.3. *Calibration graphs*

Under the optimum conditions, the various quantities of ctDNA, denaturation DNA and yRNA are used for reacting with AK, and the standard curves are plotted by ΔI values corresponding to the concentrations (see Fig. 5). The result shows that the reaction of ctDNA with AK can lead to the great enhancement of RRS intensity and the increasing RRS signal is directly proportional to the concentration of nucleic acids in the range 0.02–12.0 μg ml^{-1} for ctDNA.

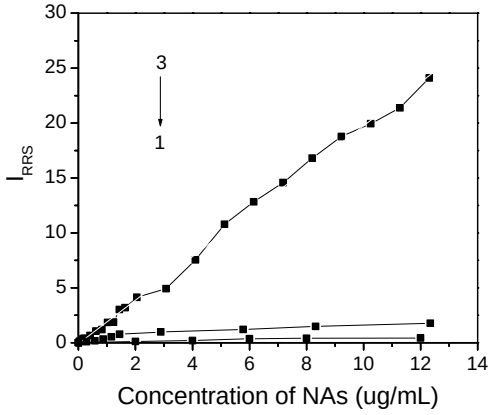

Fig. 5. RRS response for various nucleic acids: (1) denatured DNA; (2) yRNA; (3) ctDNA. Concentration of AK: 6.0 μg ml^{-1}.

The linear regression equation is $\Delta I = -0.0892 + 1.983C$ ($R = 0.9984$) and its detection limits (3σ) is 2.5 ng ml^{-1}. The method shows wide linear range and high sensitivity. However, the RRS intensities of different concentration of thermal denaturation DNA and yRNA are hardly changed.

3.4. Selectivity of the method and analytical application

The effect of foreign substances is tested under the optimal condition. The results are listed in Table 2. It can be seen from Table 2. that the permissible interfering amounts of Na^+, K^+, NH_4^+, urea, amino acid, saccharine are large. Although the permissible interfering amounts of Mn^{2+}, Ni^{2+}, Cu^{2+} are small, the allowed concentration of those interference ions is still larger than that in biological fluids. Thus, the method shows fair selectivity. To test the method, four synthetic samples containing different concentration of metal ions, amino acid, RNA and ssDNA, and two different *E. coli* plasma DNA samples are determined directly with the optimal procedure. The results are calculated according to the calibration graphs of ctDNA and listed in Tables 3 and 4, respectively. As the Table indicated, the results are satisfactory, and no significant differences between the UV-Vis and the RRS method. It also means the proposed method is suitable for the determination of dsDNA.

3.5. The reasons for enhancement of RRS

It is shown in our experiment that the RRS produced merely by the nucleic acid solution or AK solution is very faint. However, the RRS would be enhanced greatly when nucleic acids combined with the AK cation to form the reaction product. The most important reason may be a great increase in the molecular size. Under experimental conditions, AK antibiotic sulfate is dissociated and take positive charge, while nucleic acid is existed as the form of polyanion [$(NA)^{n-}$]. By the virtue of electrostatic force and hydrophobic interaction, they can combine to form supermolecule compound, in which AK molecules assemble on the nucleic acids. That is, a large number of AK molecules aggregate on the surface of nucleic acids, which made the molecular volume of the AK-ctDNA complex increased greatly. According to the law of Rayleigh scattering [31,32].

$$I = \frac{24\pi^3 N v^2}{\lambda^4} \left(\frac{{n_1}^2 - {n_0}^2}{{n_1}^2 + 2{n_0}^2} \right)^2 I_0 \quad (1)$$

where I is the intensity of scattering light, I_0 the intensity of incident light, N the number of the scattering particles per unit volume, v the volume of one particle, n_1 and n_0 are the refractive indexes of the particle and the surrounding medium, respectively. It can be seen that when the determination conditions and the concentration of the solution are fixed, the intensity of scattering is proportional to the square of the particle volume. Therefore, the increase of the molecular volume of the complex will lead to the enhancement of RRS intensity.

Table 2
Effect of foreign substances ($C_{ctDNA} = 2.0$ μg ml^{-1})

Foreign substance	Concentration (10^{-6} mol l^{-1})	Change of RRS intensity (%)	Foreign substance	Concentration (10^{-6} mol l^{-1})	Change of RRS intensity (%)
Pb^{2+}	100	0.94	Tartrate	20	−5.6
Ca^{2+}, NO_3^-	110	−7.8	Lactic acid	50	−9.0
Ni^{2+}	20	−2.2	L-leucine	250	2.9
Mn^{2+}	1.0	−6.1	Citric acid	250	−7.0
Zn^{2+}	10	−8.7	Succinic acid	100	−9.2
Mg^{2+}, SO_4^{2-}	40	−9.9	Urea	100	−8.2
Ba^{2+}, Cl^-	400	−0.75	Aminopropionic acid	100	−1.3
Al^{3+}, Cl^-	50	−2.6	Amino acetic acid	100	−7.0
Cr^{3+}, NO_3^-	10	−7.2	D-fructose	100	0.97
Fe^{3+}, Cl^-	2.8	0.48	Glucose	500	−8.2
Cu^{2+}, AC^-	2.6	−8.1	HSA[a]	0.5	−3.6
NH_4^+, Cl^-	1000	−3.6	BSA[a]	2	−7.3
Co^{2+}, Cl^-	20	−9.7	YrNA[a]	5.7	10
Na^+, Cl^-	2000	5.2	Denatured DNA[a]	6.0	5.6
K^+, Cl^-	500	7.6			

[a] μg ml^{-1}.

Table 3
The results for the determination of DNA in synthetic samples

Sample	ctDNA added ($\mu g\,ml^{-1}$)	Main additives[a]	Found value ($\mu g\,ml^{-1}$)	Recovery ($n = 5$, %)	RSD (%)
1	2.0	K^+, Al^{3+}, Fe^{3+}, Cr^{3+}, Ni^{2+}, Mg^{2+}	1.90	95.0	1.7
2	2.0	Na^+, NH_4^+, Zn^{2+}, Ca^{2+}, Cu^{2+}, Pb^{2+}	1.95	97.5	1.3
3	2.0	D-fructose, L-leucine, amino acetic acid, glucose, RNA	2.05	102.5	5.1
4	2.0	Urea, lactic acid, succinic acid, aminopropionic acid, ssDNA	2.01	100.5	1.7

[a] Concentration of additives: K^+, Na^+, $2.0 \times 10^{-6}\,mol\,l^{-1}$; Al^{3+}, Ca^{2+}, Pb^{2+}, Zn^{2+} $1.0 \times 10^{-6}\,mol\,l^{-1}$; Mg^{2+}, $8.0 \times 10^{-7}\,mol\,l^{-1}$; Ni^{2+}, $4.0 \times 10^{-8}\,mol\,l^{-1}$; Cu^{2+}, $2.6 \times 10^{-7}\,mol\,l^{-1}$; Cr^{3+}, $2.0 \times 10^{-7}\,mol\,l^{-1}$; Fe^{3+}, $5.6 \times 10^{-8}\,mol\,l^{-1}$; D-fructose, amino acetic acid, urea, succinic acid, and aminopropionic acid, $1.0 \times 10^{-5}\,mol\,l^{-1}$; L-leucine, $2.5 \times 10^{-5}\,mol\,l^{-1}$; lactic acid, glucose, $5.0 \times 10^{-5}\,mol\,l^{-1}$; RNA, ssDNA: $30\,ng\,ml^{-1}$.

Table 4
The results for the determination of DNA in *E.coli* cell extraction

Samples	UV-Vis method found ($\mu g\,ml^{-1}$)	The RRS method			
		Found value[a] ($\mu g\,ml^{-1}$)	Standard added ($\mu g\,ml^{-1}$)	Recovery value ($\mu g\,ml^{-1}$)	Recovery[b] ($n = 5$, %)
1	3.5	0.32	0		
			0.5	0.78	92.0
			1.0	1.37	104
			2.0	2.31	99.5
2	4.6	0.44	0		
			0.5	0.95	102
			1.0	1.41	97.0
			2.0	2.62	109

[a] The samples was determined by UV-Vis method, then was diluted 10 folds for determination by the proposed method.
[b] Calculations were performed using the formula: recovery[%] = 100 × (recovery value [$\mu g\,ml^{-1}$] − found value [$\mu g\,ml^{-1}$])/standard added [$\mu g\,ml^{-1}$].

4. Conclusions

Owing to the reaction of AK with dsDNA can result in the enhancement of RRS intensity, and the enhanced RRS intensity is proportion to the concentration of dsDNA in the certain ranges. Therefore, a sensitive and convenient method for the recognition and determination of dsDNA without interference from RNA and ssDNA is developed. The method can be rapidly accomplished with a common fluorescence spectrometer by using AK as the probe reagent in the weakly acidic medium. Furthermore, the proposed method has the following obvious advantages: (I) this method has a wide linear range (0.02–12.0 $\mu g\,ml^{-1}$); (II) the method is sensitive (D.L. = $2.5\,ng\,ml^{-1}$); (III) the method can be successfully applied to the determination of trace amounts of nucleic acid in practical samples; (IV) the method use the drug as probe, which hold great promise for its application in situ with dialysis; And what's more, the method is not only very sensitive for determination DNA, but also very effective to recognition dsDNA. Therefore, it would be anticipated as an effect means for recognition in vivo and determination in situ of double-stranded DNA.

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