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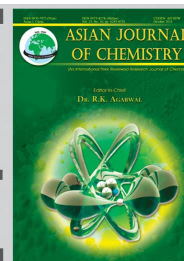

Determination of DNA at Nanogram Levels by Resonance Light Scattering Technique in Organic Acid Medium

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The different enhancement of resonance light scattering of DNA is observed in formic acid, acetic acid, propionic acid, butyric acid, trichloroacetic acid and trifluoroacetic acid. The results indicated that there was a maximum enhancement of resonance light scattering of DNA at 340 nm in 0.2 mol L^{-1} trichloroacetic acid medium. The concentration of calf thymus DNA (ctDNA) in the range of 0.05-50 μg mL^{-1} and fish sperm DNA (fsDNA) in the range of 0.05-40 μg mL^{-1} is well proportional to the enhanced resonance light scattering intensity. The detection limits are 12.2 ng mL^{-1} for ctDNA and 17.9 ng mL^{-1} for fsDNA, respectively. Nucleotides at 4 μg mL^{-1}, proteins at 0.1 μg mL^{-1} and most of metal ions at 1.0×10^{-5} mol L^{-1} do not interfere with the determination of DNAs. This method has been applied to the determination of calf thymus DNA and fish sperm DNA in synthesized samples with satisfactory results.

Key Words: Resonance light scattering, DNA, Organic acid, Trichloroacetic acid.

INTRODUCTION

Nucleic acid is the essential genetic material, which plays an important role in the life science. The research of nucleic acid is very important in biology, biochemistry and medicine field. In the chemistry research field of nucleic acid, it has been very active that the small molecules is used as anticancer drugs, the dye of nucleic acid and construction probe. Resonance light scattering (RLS) is a new analytical technology developed in last ten years[1,2]. For its remarkable characteristics of high sensitivity, high selectivity and simple operation, this method received increasingly attention and has been applied widely to the study of biological macromolecules such as nucleic acids[3], proteins[4] and saccharide[5] and determination of trace metal ions[6], nonmetal ions[7], nanoparticle[8] and some pharmaceuticals[9].

In organic acid (formic acid, acetic acid, propionic acid, butyric acid, trichloroacetic acid and trifluoroacetic acid) solution, the native double-helical nucleic acids will be denatured, then the denatured nucleic acids can aggregate to form large particles. The size of the particles is the same as that of ultraviolet visible light wave[10]. In this study, the enhanced light scattering intensity of DNA in different organic acid solution was measured. It was demonstrated that the resonance light scattering intensity of DNA in trichloroacetic acid (TCA) was the highest and most stable. The enhanced light scattering intensity of DNA in trichloroacetic acid medium was well proportional to the concentration of nucleic acids (calf thymus DNA and fish sperm DNA). Based on this, a new method for the determination of DNA by resonance light scattering technique was developed. This method has many advantages, such as simple, sensitive and economical and easy to perform.

EXPERIMENTAL

A Hitachi F-4500 fluorescence spectrophotometer (Tokyo, Japan) equipped with a quartz cell (1 × 1 cm) was used to record the spectra of resonance light scattering and to measure the intensity at a given wavelength (PMT voltage, 400 V; slit (ex/em), 10/10 nm). A Simplicity Ultrapure Water System (Millipore Co., USA) was used to make ultra-pure water.

The stock solution (10 mol L^{-1}) of formic acid, acetic acid, propionic acid, butyric acid (A.R. Medicine Company of Tianjin, China) were prepared by solution in water; the stock solution (2 mol L^{-1}) of trichloroacetic acid (A.R. Chemical Reagent Co. of Shanghai, China) and trifluoroethyl acid (HPLC Tedia Company Inc., USA) were prepared by solution in water. The stock solution (100 mg mL^{-1}) of calf thymus DNA (ctDNA, Xin-Jing-Ke Biochemical Reagent Co. China) and fish sperm DNA (fsDNA, Xin-Jing-Ke Biochemical Reagent Co., China) were prepared by dissolving in water and store in a refrigerator at 0-4 ºC. Working solutions (20 mg mL^{-1}) were obtained by

dilution the stock solutions with water immediately prior to use. Britton-Robinson (BR) buffer was used in the experiments. All other chemicals used were of analytical grade and ultra-pure water was used throughout.

Methods: In a 10 mL volumetric flask, a known volume of standard DNA solution or sample solution was transferred in and 7 mL ultra-pure water and then 1 mL trichloroacetic acid solution was added. This mixture was finally diluted to 10 mL with ultra-pure water and mixed thoroughly and put for 5 min. The resonance light scattering spectrum was obtained by scanning simultaneously with the same excitation and emission wavelength. The resonance light scattering intensity was measured at 340 nm with slit width at 10 nm for both excitation and emission. In this paper, the resonance light scattering signals of DNA in organic acid solution are net intensity of resonance light scattering in arbitrary units of the instrument, where the background signal of organic acid solution in the absence of DNA has been subtracted for each value except for resonance light scattering spectra.

RESULTS AND DISCUSSION

Spectral characteristics: Figs. 1 and 2 showed that the resonance light scattering signals of ctDNA is also strongly enhanced at different concentration of levels formic acid, acetic acid, propionic acid, butyric acid, trichloroacetic acid and trifluoroacetic acid solution. The resonance light scattering reached a maximum at 340 nm and speedily decreases with the increase of the wavelength. The results showed that the order of the maximum light scattering intensities of ctDNA in various organic acid solution is trichloroacetic acid ≈ trifluoroacetic acid > formic acid > acetic acid > propionic acid > butyric acid, which is accordance with the order of their acidic strength. Subsequent experiments showed that the fsDNA have the almost same resonance light scattering intensity with ctDNA in different acid medium, which indicated that there was not significant difference in the aggreative function of different kinds of DNA in organic acid solution.

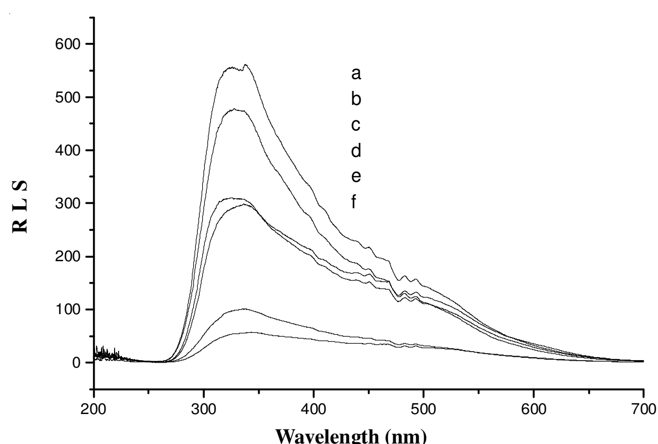

Fig. 1. Resonance light scattering spectra of ctDNA (2.0 mg mL^{-1}) in acetic acid, propionic acid, butyric acid solution (5.0 mol L^{-1}). a, ctDNA-butyric acid; c, ctDNA-propionic acid; d, ctDNA-acetic acid; b, 5 butyric acid; e, propionic acid; f, acetic acid

Effects of the concentration of organic acid: The effects of organic acid concentration on the enhanced resonance light scattering intensity of ctDNA were shown in Figs. 3 and 4. With the increase of organic acid concentration, the light scattering intensity of ctDNA greatly increased at first and then gradually decreased. The enhanced intensity in formic acid, trichloroacetic acid and trifluoroacetic acid were higher that in others.

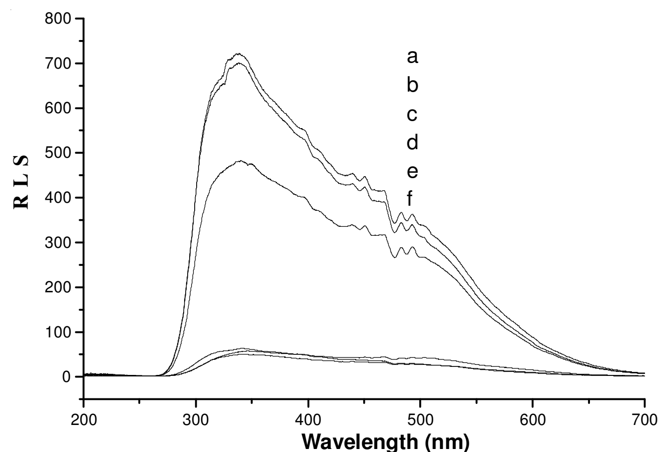

Fig. 2. Resonance light scattering spectra of ctDNA (2.0 mg mL^{-1}) in 0.2mol L^{-1} trichloroacetic acid, 0.05 mol L^{-1} trifluoroacetic acid and 5.0 mol L^{-1} formic acid. a, ctDNA-trichloroacetic acid; b, ctDNA-trifluoroacetic acid; c, ctDNA-formic acid; d, trichloroacetic acid; e, formic acid; f, trifluoroacetic acid

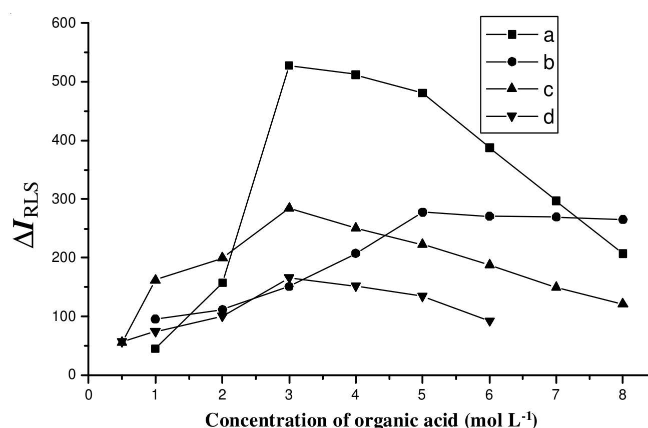

Fig. 3. Effect of formic acid, acetic acid, propionic acid and butyric acid concentration on the enhanced resonance light scattering intensity of ctDNA (2.0 mg mL^{-1}). a, formic acid; b, acetic acid; c, propionic acid; d, butyric acid

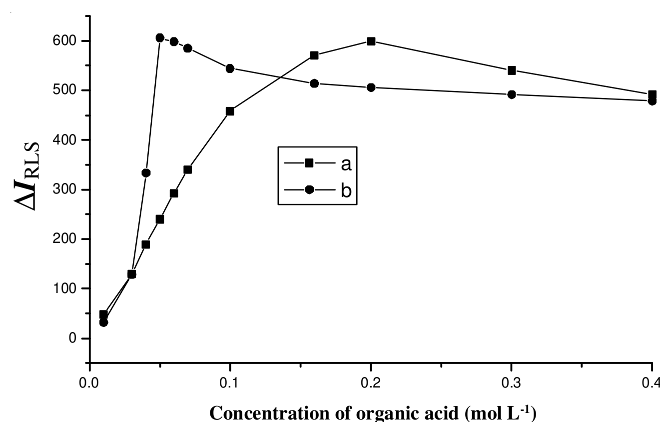

Fig. 4. Effect of trichloroacetic acid, trifluoroacetic acid concentration on the enhanced resonance light scattering intensity of ctDNA (2.0 mg mL^{-1}). a, trichloroacetic acid; b, trifluoroacetic acid

TABLE-1
INFLUENCE OF FOREIGN SUBSTANCES

Substance	Coexisting concentration	Change of I_{RLS} (%)	Substance	Coexisting concentration	Change of I_{RLS} (%)
BSA	0.1 μg mL^{-1}	1.6	Cu^{2+}, SO_4^{2-}	1×10^{-5} mol L^{-1}	-3.4
AMP	4 μg mL^{-1}	-1.2	Co^{2+}, Cl^-	1×10^{-5} mol L^{-1}	-3.0
CMP	4 μg mL^{-1}	-3.4	Ni^{2+}, NO_3^-	1×10^{-5} mol L^{-1}	-0.88
TMP	4 μg mL^{-1}	-3.5	Fe^{2+}, SO_4^{2-}	1×10^{-5} mol L^{-1}	-2.9
Ca^{2+}, Cl^-	1×10^{-2} mol L^{-1}	-21.3	Al^{3+}, SO_4^{2-}	1×10^{-6} mol L^{-1}	-22.4
Ca^{2+}, Cl^-	1×10^{-3} mol L^{-1}	0.8	Al^{3+}, SO_4^{2-}	1×10^{-7} mol L^{-1}	-4.7
Mg^{2+},SO_4^{2-}	1×10^{-2} mol L^{-1}	-1.3	Fe^{3+}, Cl^-	1×10^{-6} mol L^{-1}	-67.9
Mg^{2+},SO_4^{2-}	1×10^{-3} mol L^{-1}	-0.32	Fe^{3+}, Cl^-	1×10^{-7} mol L^{-1}	-2.8
Zn^{2+}, Cl^-	1×10^{-5} mol L^{-1}	4.6	Hg^{2+} NO_3^-	1×10^{-5} mol L^{-1}	-1.8
Cd^{2+}, Cl^-	1×10^{-5} mol L^{-1}	3.9	Pb^{2+}, NO_3^-	1×10^{-5} mol L^{-1}	2.5
Mn^{2+}, SO_4^{2-}	1×10^{-5} mol L^{-1}	0.88	Cr^{3+}, NO_3^-	1×10^{-5} mol L^{-1}	-1.7

BSA: bovine serum albumin; AMP: adenosine-5'-phosphote; TMP: thymine-5'-phosphote; CMP: cytidine-5'-phosphote

Effects of time: By using resonance light scattering signal derived from particles of DNA incubation time, which affect the stability of the resonance light scattering signal, is a main factor to affect the accuracy and precision of the method. Fig. 5 shows the maximum of the light scattering intensity of ctDNA was obtained at 3 min for trichloroacetic acid solution, 5 min for trifluoroacetic acid solution and 10 min for formic acid solution, respectively, which indicated that the action of aggregation of DNA was rapid and could form larger particles in short period of time. The results showed that the stability of the resonance light scattering signal of DNA was poor in formic acid and in trifluoroacetic acid, the resonance light scattering signal decreased greatly with the prolongation of time. But the resonance light scattering signal in trichloroacetic acid solution there was no significant difference in the period of 180 min. The reason may be ascribed to the different particle characteristics of nucleic acids in trichloroacetic acid and in other acid medium (HCl, H_2SO_4, HNO_3, HCOOH, CF_3COOH). It is likely that the nucleic particles formed in trichloroacetic acid solution could keep a constant size. The results showed that this technique was a quite effective method for the determination of DNA.

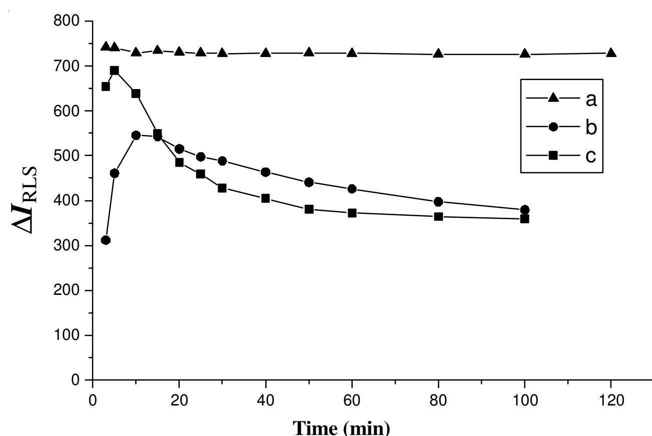

Fig. 5. Effect of incubation time on the enhanced resonance light scattering of ctDNA (2.0 mg mL^{-1}) in trichloroacetic acid, formic acid and trifluoroacetic acid solution. a, 0.2 mol L^{-1} trichloroacetic acid; b, 3 mol L^{-1} formic acid; c, 0.05mol L^{-1} trifluoroacetic acid

Effects of ionic strength: Fig. 6 shows the influence of ionic strength on the enhanced resonance light scattering intensity of ctDNA in 0.2 mol L^{-1} trichloroacetic acid solution. The light scattering intensity remained constant when NaCl concentration was between 0-0.1mol L^{-1}. The reason was that the acidity of trichloroacetic acid was such strong that 0.1 mol L^{-1} Na^+ had not any influence on the denaturation of large practicles of DNA.

Interference: The tolerance concentration of coexisting substances including protein, nucleticles and metal irons was investigated by premixing 2 μg mL^{-1} ctDNA with the foreign substances and determining its light scattering signal according to the standard procedure. The results were listed in Table-1, which showed that protein can be allowed at 0.1 mg mL^{-1}, while the nucleotides do not interfere the assay at 4 mg mL^{-1}. Most of metal ions can be tolerated at high concentration (1×10^{-2} mol L^{-1}-1×10^{-5} mol L^{-1}) except Fe^{3+} and Al^{3+}. The tolerable concentration of Fe^{3+} and Al^{3+} is generally higher than those exsisted in biological samples or PCR products. Therefore, it is characterized that the resonance light scattering assay is very practical.

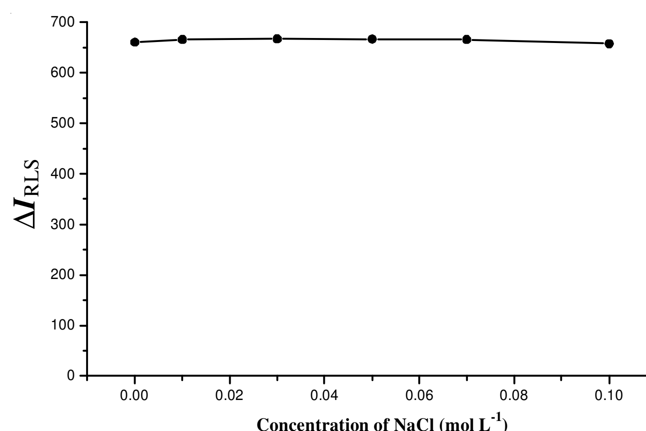

Fig. 6. Influence of ionic strength on the enhanced resonance light scattering of ctDNA (2.0 mg mL^{-1}) in 0.2 mol L^{-1} trichloroacetic acid solution

Calibration graphs of DNA and determination of synthetic samples: According to the general procedures, the linear range, linear regression equations, detection limits and correlation coefficient (R) for determination of ctDNA and fsDNA were obtained and summarized in Table-2. It was proved that there are good relationships between the enhanced

TABLE-2
ANALYTICAL PROPERTIES OF THE RLS METHOD

Nucleic acids	Linear range (μg mL^{-1})	Linear regression equation (C, μg mL^{-1})	Detection limit (3σ, ng mL^{-1})	R
Calf thymus DNA	0.05-3	$\Delta I = -4.23 + 321.1C$	12.2	0.9987
	3-50	$\Delta I = 723.1 + 110.7C$		0.9991
Fish sperm DNA	0.05-3	$\Delta I = -11.9 + 310.1C$	17.9	0.9993
	3-40	$\Delta I = 710.3 + 110.3C$		0.9983

C_{TCA} = 0.2 mol L^{-1}

TABLE-3
DETERMINATION OF SYNTHETIC SAMPLES

Nucleic acids	Conc. (μg mL^{-1})	Coexisting substances	Mean found (μg mL^{-1})	Recovery (n=5) (%)	RSD (n = 5) (%)
Calf thymus DNA	2.50	BSA, Fe^{3+}, Ca^{2+}, Mg^{2+}	2.55	101.2-102.8	4.24
Calf thymus DNA	2.50	AMP, CMP, TMP	2.53	99.4-102.3	3.11
Fish sperm DNA	2.50	BSA, Al^{3+}, Ca^{2+}, Mg^{2+}	2.57	100-103.2	5.70
Fish sperm DNA	2.50	AMP, CMP, TMP	2.52	98.7-101.1	5.35

Concentration of BSA is 0.1 μg mL^{-1}, AMP, CMP, TMP is 2.5 μg $mL^{-1,}$ Fe^{3+} and Al^{3+} is 1×10^{-7}mol L^{-1}; Ca^{2+},Mg^{2+} is 1×10^{-4}mol L^{-1}

resonance light scattering intensity and the concentration of nucleic acids in a wide range. The RSDs for eleven replicate experiments of 0.05 μg mL^{-1} CtDNA in 0.2 mol L^{-1} trichloroacetic acid solution was 2.5 %.

To examine the applicability and accuracy of this method, four synthetic samples of ctDNA and fsDNA containing metal ions, bases and proteins were determined by the proposd method. The results were listed in Table-3, which indicate that DNA in synthetic samples can be determined with satisfactory results. It also worth noting that the determination in 0.2 mol L^{-1} trichloroacetic acid medium has a good precision.

In organic acid solution, nucleic acid can aggregate to form large particles, which can result in the enhance ment of the resonance light scattering signal intensity. Based on this, a novel sensitive resonance light scattering method has been developed for the determination of DNA. Compared with other reported methods, this method is convenient, rapid, inexpensive and simple. The method has been applied to the assay of DNA in synhetic samples with satisfactory results. Trichloroacetic acid can quantitatively precipitate DNA. So the proposed method is likely promising for sensitive determination of DNA extracted from biological sample, or PCR product.

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