$[Ru(phen)_2(dppz)]^{2+}$ Assemble on the Surface of the SDS Micelle and Its Application for the Determination of DNA

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The solution of $[Ru(phen)_2(dppz)]^{2+}$ and SDS has high Resonance Light Scattering (RLS) signals due to $[Ru(phen)_2(dppz)]^{2+}$ assemble on the surface of the SDS micelle. Because of the high affinity ($K_B \geq 10^6$ L mol^{-1}) between $[Ru(phen)_2(dppz)]^{2+}$ and DNA, the adding of DNA in the solution of $[Ru(phen)_2(dppz)]^{2+}$-SDS makes the dissociation of $[Ru(phen)_2(dppz)]^{2+}$-SDS, and results in decreasing of the RLS signals and increasing of the absorbance. Based on this, a novel method is proposed for DNA assay. Under optimum condition, good linear relationship was obtained within the concentration range of 0.018-1.26 μg mL^{-1}, the linear equation is I_{RLS} = 504.8-348.8 c (c: μg mL^{-1}) and the correlation coefficient (r) is 0.9992. The detect limit for calf thymus DNA is 8.6 ng mL^{-1}. The proposed method was successful applied to determine the extracted *colibacillus* plasmid DNA.

Key Words : $[Ru(phen)_2(dppz)]^{2+}$, SDS, DNA, Resonance light scattering

Introduction

Over the past decade there has been substantial interesting in the binding properties of a number of ruthenium(II) complexes. One of the most interesting observations is the discovery of the molecular "light switch" complexes,[1] which are not photoluminescent in water but emit in nonaqueous solvents or in the presence of DNA. Up to now, many "light switch" complexes have been founded, such as $[Ru(phen)_2(dppz)]^{2+}$, $[Ru(bipy)_2(dppz)]^{2+}$, $[Ru(5,6\text{-}dmp)_2(dppz)]^{2+}$,[2] $[Ru(bipy)_2(PHEHAT)]^{2+}$ [3] and $[Ru(pztp)_2(phen)]^{2+}$ [4] *etc.* Some polypyridyl Os(II) complexes, such as $[Os(phen)_2(dppz)]^{2+}$ also have been found act as "light switch" for DNA.[5] The studies about molecular "light switch" complexes focused on three aspects: 1. Searching for new molecular "light switch" complexes;[2-5] 2. Depicting the change tendency and the fluorescence intensity of molecular "light switch" complexes when they interact with DNA and investigating the interaction mode between molecular "light switch" complexes and DNA;[6-10] 3. Studying the properties and the utility of molecular "light switch", such as quantitative determination of DNA,[11,12] detection of mismatches,[13] triplex DNA study,[14] signaling protein-DNA binding[15] and determination of low concentration of water in nonaqueous solution[16] *et al.*.

Light scattering is a major source of interference in common spectrofluorometry. But based on the enhancement of RLS signals, many Resonance Light Scattering (RLS) methods were established for DNA assay in recent years.[17,18] The RLS methods for the determination of DNA using some molecular "light switch" complexes also have been established in our previous study.[19] But few methods have been reported to determine DNA based on the decreasing of RLS. Hereby the interaction between $[Ru(phen)_2(dppz)]^{2+}$ and DNA in the presence of SDS has been studied by the RLS spectra and an interesting phenomena of decrease of RLS signals by adding DNA in $[Ru(phen)_2(dppz)]^{2+}$-SDS system was observed. Based on this, a novel method is established for DNA assay.

Experimental Section

Apparatus. The light scattering spectra and intensity of light scattering were measured by a Perking Elmer Model LS-55 spectrometer with a quartz cuvette (10 × 10 mm). A Shimadzu Model UV-1601 double-beam spectrophotometer was used for recording the absorption spectra. The pH was measured with a Model pHS-3C meter (Shanghai Leici Equipment Factory, China).

Reagents. All chemicals were analytical reagents of the best grade commercially available. All stock solutions were prepared using doubly distilled water. The calf thymus DNA was purchased from HuaMei biochemical Co. $Ru(phen)_2(dppz)(BF_4)_2 \cdot 2H_2O$ were synthesized according to reference[1] and identified by ^{1}H NMR.[11] The ^{1}H NMR of $[Ru(phen)_2(dppz)]^{2+}$ (in CD_3COCD_3) found δ 9.70 (2H, m), δ 8.73 (4H, m), δ 8.50 (2H, m), δ 8.37-8.25 (8H, m), δ 8.20 (2H, dd), δ 8.07 (2H, dd), δ 7.79 (2H, m), δ 7.72 (4H, m).

Preparation of extracted plasmid DNA. The following procedure for plasmid DNA extraction was recommended: An amount of 1.5 mL of overnight bacteria culture collected into a microtube was centrifugalized at 12,000 rpm at 4 °C for 30 sec, the supernatant was removed as completely as possible by a Pasteur pipette. Dissolved the bacteria in 0.1 mL of solution A (50 mmol L^{-1} glucose, 25 mmol L^{-1} Tris-HCl, pH = 8.0, 10 mmol L^{-1} EDTA, 0 °C). Then a 0.2 mL of

solution B (0.2 mol L^{-1} NaOH£¯1% SDS) was added to break the cells and denatured the nucleic acids. After the tube was put on ice for about 4 min, 0.15 mL of solution C (3 mol L^{-1} KAc, 11.5% HAc) was added to make the neutralization of the solution pH. The tube was centrifugalized at 12,000 rpm for about 8 min, the plasmid DNA would remind in solution, whereas the chromosomal DNA and most of the cellular RNA and protein would precipitate. Transferred around all of the supernatant to a fresh tube, equal volume of chloroform/isoamyl alcohol (1 : 1 mixture) was added and mixed completely. After the suspension was centrifugalized at 12,000 rpm for 8 min, the upper phase (containing the nucleic acids) was carefully transferred to a fresh tube, and 1ml of 100% ethanol was added. Stand the tube at room temperature for 10 min, then precipitated the nucleic acids by centrifuge at 12,000 rpm for 10 min. The supernatant was removed as completely as possible and the pellet (composed of plasmid DNA and some small molecular RNA) was dried at 65 °C for about 2 min, then 0.25 mL of TE buffer (10 mmol L^{-1} Tris-HCl, 1 mmol L^{-1} EDTA, pH = 8.0) was added to dissolve the sample.

Procedures. In a 10.0 mL volumetric flask, appropriate concentration of $[Ru(phen)_2(dppz)]^{2+}$, SDS, DNA and BR Buffer were added and diluted to 10.0 mL with doubly distilled water. The above solutions were kept in 1 cm quartz cuvettes and were measured 30 minutes after the samples were mixed. The light scattering spectrum was obtained by scanning simultaneously with the same excitation and emission wavelengths.

Results and Discussion

Features of RLS spectra. The RLS spectra of molecular "light switch" are shown in Figure 1. Reagent blank RLS signals of $[Ru(phen)_2(dppz)]^{2+}$ (Fig. 1a), DNA (Fig. 1b) and SDS (Fig. 1c) are low. Both the mixture of SDS and DNA and the mixture of $[Ru(phen)_2(dppz)]^{2+}$ and DNA have low RLS signals (not shown in Fig. 1). However, the mixture of $[Ru(phen)_2(dppz)]^{2+}$ and SDS has high RLS intensity due to $[Ru(phen)_2(dppz)]^{2+}$ assemble on the surface of the SDS micelle (Fig. 1e). But when DNA was added, the RLS signals decrease greatly (Fig. 1d), which may be due to that $[Ru(phen)_2(dppz)]^{2+}$ has stronger interaction with DNA by intercalating into the base pairs of DNA with the binding constant more than 10^6 L mol^{-1}.[1] So SDS-$[Ru(phen)_2(dppz)]^{2+}$ was dissociated and resulted in decreasing of RLS signals.

It was found that the decrease of RLS signals of $[Ru(phen)_2(dppz)]^{2+}$-SDS-DNA system is in proportion to the concentration of DNA and could be used to determine DNA. Among three peaks of 336 nm, 393 nm and 480 nm, peak at 336 nm is corresponding to the absorbance valley between two absorbance peaks at 373 nm and 263 nm which changes greatly by adding SDS or DNA (Fig. 2), so it is selected for the following study.

Absorbance spectra study. Another interesting phenomenon was observed in the absorption spectra of this system (Fig. 2). Previous study showed that strong hypochromic effect and red shift appeared with the absorption spectra of $[Ru(phen)_2(dppz)]^{2+}$, which owing to $[Ru(phen)_2(dppz)]^{2+}$ intercalate into the base pairs of DNA.[20-22] However, in this system, the absorbance value of $[Ru(phen)_2(dppz)]^{2+}$ decreased greatly in the presence of SDS (Fig. 2a and d), and when DNA was added, the hyperchromic effect instead of hypochromic effect was observed (Fig. 2b and c). These reveal that SDS brings stronger hypochromic than DNA. Previous study shows that the absorbance peak at 263 nm of $[Ru(phen)_2(dppz)]^{2+}$ decreased by about 11.2% in the presence of 35.0 μg mL^{-1} DNA,[20] while it decreased by about 50% in the presence of 2.5×10^{-4} mol L^{-1} SDS (Fig. 2d). The study of absorption spectra is consistent with the results of the RLS spectra study. $[Ru(phen)_2(dppz)]^{2+}$ could assemble on the surface of the SDS micelle and results in strong hypochromic effect, but when DNA was added,

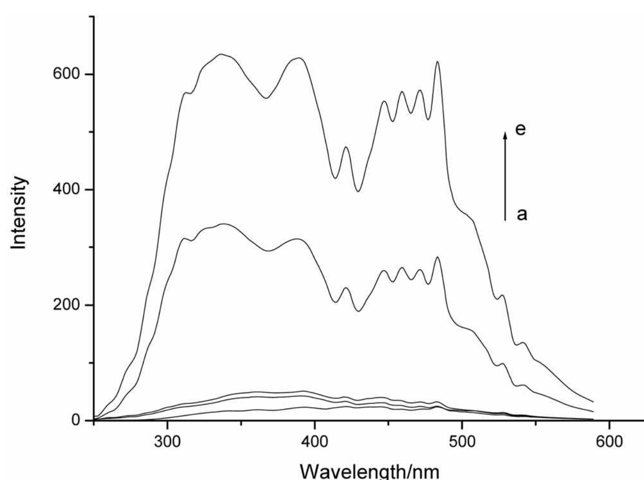

Figure 1. RLS spectra of a. $[Ru(phen)_2(dppz)]^{2+}$, b. DNA, c. SDS, d. $[Ru(phen)_2(dppz)]^{2+}$+SDS+DNA and e. $[Ru(phen)_2(dppz)]^{2+}$ + SDS $[Ru(phen)_2(dppz)]^{2+}$: 4.0×10^{-6} mol L^{-1}, SDS: 2.5×10^{-4} mol L^{-1}, DNA: 0.80 μg mL^{-1}.

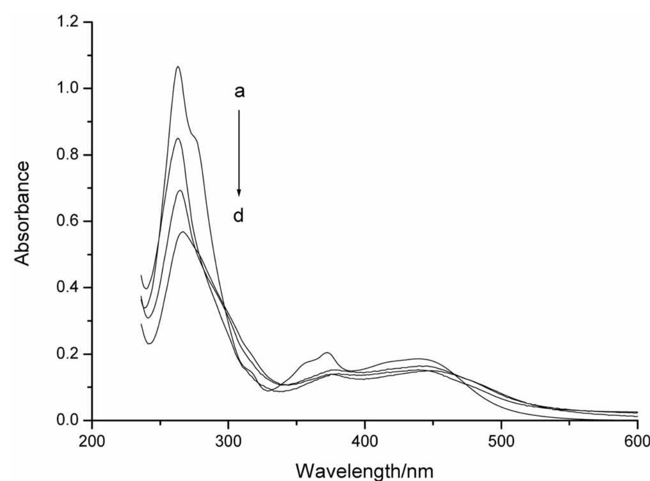

Figure 2. Absorbance spectra of a. $[Ru(phen)_2(dppz)]^{2+}$, b. a+SDS+2.0 μg mL^{-1} DNA, c. a+SDS+1.0 μg mL^{-1} DNA, d. a+SDS $[Ru(phen)_2(dppz)]^{2+}$: 1.0×10^{-5} mol L^{-1}, SDS: 2.5×10^{-4} mol L^{-1}.

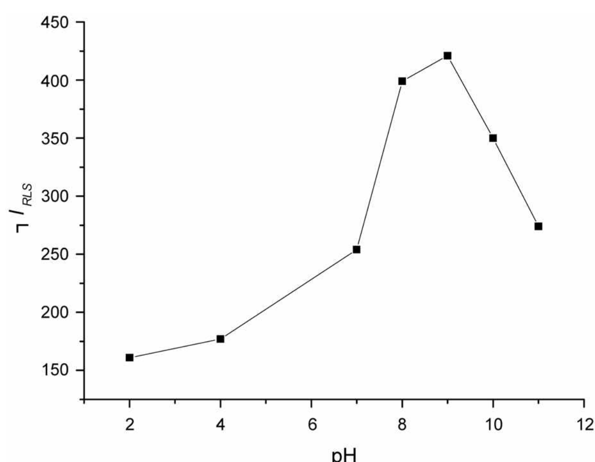

Figure 3. Effect of pH on the change of RLS signals. $[Ru(phen)_2(dppz)]^{2+}$: 4.0 × 10^{-6} mol L^{-1}, SDS: 2.5 × 10^{-4} mol L^{-1}, DNA (1.20 μg mL^{-1}).

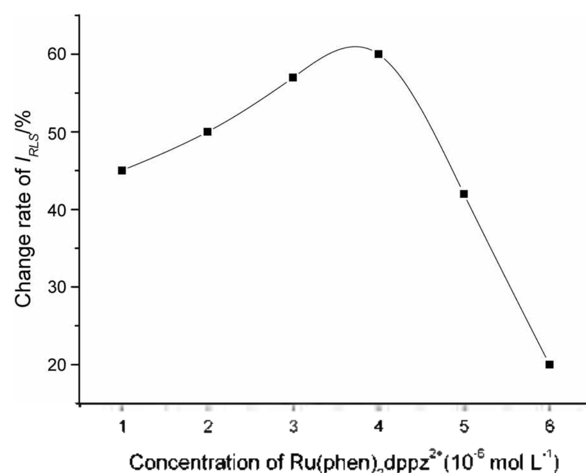

Figure 5. Influence of the concentration of $[Ru(phen)_2(dppz)]^{2+}$ on the change rate of RLS signals by adding DNA. SDS: 2.5 × 10^{-4} mol L^{-1}, DNA: 0.80 μg mL^{-1}, pH 9.0.

$[Ru(phen)_2(dppz)]^{2+}$ was apart from SDS and intercalate in to the base pairs of DNA, then the absorbance increased.

Optimization of conditions for the determination of DNA.

***pH effect*:** The effect of pH on the change of RLS intensity (ΔI_{RLS}) of the system by adding DNA was investigated during the range of 2.0-11.0 (Fig. 3). The result indicates that the ΔI_{RLS} increased as the pH increased until at pH 9.0, the maximum value of ΔI_{RLS} is reached. Above pH 9.0 ΔI_{RLS} decreased.

***Effect of the concentration of SDS*:** The concentration of SDS is one of the most important conditions to observe the phenomena of decreasing of RLS signals by adding DNA. Only when the SDS concentration within the range from 5.0 × 10^{-5} mol L^{-1} to 5.0 × 10^{-4} mol L^{-1}, decrease of RLS signals can be observed. When the concentration of SDS is lower than 5.0 × 10^{-5} mol L^{-1}, no apparent increasing of the RLS signals occurs by adding SDS into $[Ru(phen)_2(dppz)]^{2+}$ solution, which owning to the micelle of SDS are not large enough to bring high RLS signals. Correspondingly, no decreasing of RLS signals was observed by adding DNA. However, the RLS signals will increase slightly by adding DNA while the SDS concentration is higher than 5.0 × 10^{-4} mol L^{-1}, which may due to high concentration of SDS will prevent $[Ru(phen)_2(dppz)]^{2+}$ to biding DNA.[23] Figure 4 show the effect of the concentration of SDS during the range from 1.0 × 10^{-4} mol L^{-1} to 3.8 × 10^{-4} mol L^{-1}, the maximum change of RLS signals occurs while the concentration is 2.5 × 10^{-4} mol L^{-1}.

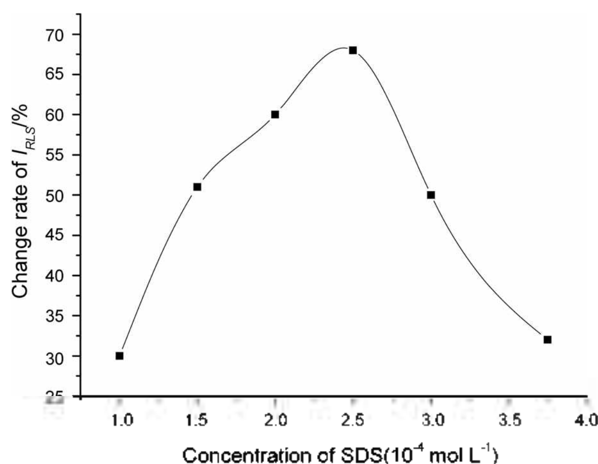

Figure 4. Influence of the concentration of SDS on the change rate of RLS signals by adding DNA. $[Ru(phen)_2(dppz)]^{2+}$: 4.0 × 10^{-6} mol L^{-1}, DNA: 0.80 μg mL^{-1}, pH 9.0.

***Effect of the $[Ru(phen)_2(dppz)]^{2+}$ concentration*:** $[Ru(phen)_2(dppz)]^{2+}$ concentration between 1.0 × 10^{-6} mol L^{-1} and 6.0 × 10^{-6} mol L^{-1} does not interfere the phenomena of decreasing of RLS signals by adding DNA, and the RLS signals change greatly by adding 0.80 μg mL^{-1} ctDNA while the concentration of $[Ru(phen)_2(dppz)]^{2+}$ is 4.0 × 10^{-6} mol L^{-1} (Fig. 5). Therefore, 4.0 × 10^{-6} mol L^{-1} was used in the subsequent study.

***Effect of the ionic strength*:** NaCl was used to control the ionic strength of the solution. The results show that the change of RLS intensity decreased with the increasing of ionic strength from 0.001 M to 0.1 M, which due to reaction between $[Ru(phen)_2(dppz)]^{2+}$ and SDS would be restrained with the increasing of ionic strength and result in decreasing of the change of RLS intensity by adding DNA.

***Effect of reaction time*:** The experiments show that the light-scattering signal reached a constant value within 30 min after DNA had been added and remained stable up to 90 min. So the measurements could be done within 30-90 min.

***Influence of the addition order of reagent*:** Different kinds of addition order of reagents were investigated. The result show that the addition order of reagents affects the RLS intensity of the system and the best order is B-R buffer, $[Ru(phen)_2(dppz)]^{2+}$, SDS and DNA. The results also estimate the model proposed before. That is, $[Ru(phen)_2(dppz)]^{2+}$ could assemble on the surface of the SDS micelle and they were dissociated by adding DNA.

***Influence of coexisting substances*:** The influence of

Table 1. Tolerance of foreign substances. (pH = 9.0, $[Ru(phen)_2(dppz)]^{2+}$: 4.0×10^{-6} mol L^{-1}, SDS: 2.5×10^{-4} mol L^{-1}, DNA: 0.80 μg mL^{-1})

Foreign substance	Concentration (μg mL^{-1})	Change of ΔI_{RLS}(%)
Ca^{2+} (Cl^-)	20	1.6
Mg^{2+} (Cl^-)	20	1.0
Na^+ (Cl^-)	20	0.1
Mn^{2+} (Cl^-)	20	6.2
Al^{3+} (Cl^-)	5	5.0
EDTA	10	3.7
BSA	5	−1.0
Lactose	20	−8.6
Citric acid	20	−3.0
Glutamic acid	10	6.8
Thymine	20	−3.8
Uracil	20	−5.2
Guanine	20	3.4
Sucrose	20	−2.9

Table 2. Recovery of ctDNA in extracted samples. (n = 3) (pH = 9.0, $[Ru(phen)_2(dppz)]^{2+}$: 4.0×10^{-6} mol L^{-1}, SDS: 2.5×10^{-4} mol L^{-1})

Sample	plasmid DNA (μg mL^{-1})	ctDNA		Recovery (%)
		Added (μg mL^{-1})	Found (μg mL^{-1})	
1	0.10	0.40	0.427	107
2	0.30	0.40	0.415	104
3	0.50	0.40	0.384	96

various ions, bases, proteins and other biochemical reagents was tested. When the concentration of ctDNA was 0.80 μg mL^{-1} under the optimal condition. The interference levels are summarized in Table 1. The results indicate that ions, bases, and proteins do not interfere with the determination of nucleic acids in the proposed method.

Calibration and assay of extracted samples. The decrease of RLS signals of $[Ru(phen)_2(dppz)]^{2+}$-SDS-DNA system is in proportion to the concentration of DNA during the range of 0.018-1.26 μg mL^{-1}. Under the optimum conditions, the linear regression equation of ctDNA is I_{RLS} = 504.8-348.8 *c* (*c*: μg mL^{-1}) and the correlation coefficient is 0.9992, and the detect limit for ctDNA is 8.6 ng mL^{-1}.

The proposed method was successful applied to determine the extracted *colibacillus* plasmid DNA. The addition and recovery of ctDNA in extracted samples were also studied to detect the applicability of the method. As shown in Table 2, the values found for the three samples are identical with the expected ones, the recoveries are within 96-107%.

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