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Study on the interaction between nucleic acid and Eu^{3+}–oxolinic acid and the determination of nucleic acid using the resonance light scattering technique

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Abstract

At pH 9.75, the resonance light scattering (RLS) intensity of OA–Eu^{3+} system is greatly enhanced by nucleic acid. Based on this phenomenon, a new quantitative method for nucleic acid in aqueous solution has been developed. Under the optimum condition, the enhanced RLS is proportional to the concentration of nucleic acid in the range of 1.0×10^{-9} to 1.0×10^{-6} g/ml for herring sperm DNA, 8.0×10^{-10} to 1.0×10^{-6} g/ml for calf thymus DNA and 1.0×10^{-9} to 1.0×10^{-6} g/ml for yeast RNA, and their detection limits are 0.020, 0.011 and 0.010 ng/ml, respectively. Synthetic samples and actual samples were satisfactorily determined. In addition, the interaction mechanism between nucleic acid and OA–Eu^{3+} is also investigated.

Keywords: Nucleic acids; Oxolinic acid; Resonance light scattering

1. Introduction

The quantitative analysis for micro amounts of nucleic acids is required in many fields such as biochemistry, molecular biology, biotechnology, and medical diagnostics. Since using the intrinsic fluorescence and ultraviolet absorption of nucleic acids for their determination has been severely limited by low sensitivity and serious interference [1]. Therefore, some probes based on the interaction between nucleic acids and extrinsic reagents have been employed in the determination of nucleic acids using the spectral methods. Among these spectral methods, resonance light scattering (RLS) technique has given rise to strong interest among chemists and biochemists since Pasternack et al. pioneering work [2,3] using a common spectrofluorometer. Huang et al. first used this technique for analytical purposes to determine trace amounts of nucleic acids [4,5]. Subsequently, more studies of quantitative determination of macromolecules including nucleic acids [6–8] and proteins [9,10] and high sensitivity detection of DNA hybridization [11] by RLS have been published. Up to now, most of the probe applied in the determination of nucleic acids are organic dyes [12,13] by their aggregation on DNA, these methods used above probe are sensitive, inexpensive and safe, but they also have the disadvantage of easy to be interfered by foreign ions and narrow linear range.

In this paper, our focus is to develop the complex of Eu^{3+} and oxolinic acid (OA) as a sensitive RLS probe for the determination of trace amount of nucleic acids. OA is one of quinolone antibiotics used in treating bacterial diseases in aquatic species. Eu^{3+}–OA complex and nucleic acid have drastic interaction. The detection limit reaches 10^{-11} g/ml for nucleic acids. And its high ability to tolerance foreign ions and broad linear range also overcome the limitation on selectivity and narrow linear range of other method used dye. So study this question is important since it not only improved the

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detection limit of nucleic acid but also developed the applied range of RLS.

2. Experimental

2.1. Apparatus

The RLS spectra and the intensity of RLS were measured with a F-4500 spectrofluorimeter (Hitachi, Japan). All absorption spectra were measured on a UV-4100 spectrophotometer (Hitachi, Japan). All pH measurements were made with a Delta 320-S pH meter (Mettler Toledo).

2.2. Chemicals

Stock solutions of nucleic acids (100 μg/ml) were prepared by dissolving commercially purchased herring sperm DNA (fsDNA, Sigma, Germany), calf thymus DNA (ctDNA, Beijing Baitai Reagent Company, Beijing, China), and yeast RNA (yRNA, Sigma, Germany) in doubly deionized water. Working standard solutions were obtained by appropriate dilution of the stock solutions.

Stock standard solution of Eu^{3+} (1.0×10^{-2} mol/l) was prepared by dissolving the corresponding oxide (99.9%) in hydrochloric acid and heating until nearly dry then diluting with doubly deionized water.

Stock solution of OA (5.0×10^{-3} mol/l) was prepared by dissolving the appropriate amount of OA with 0.1 mol/l NaOH, and diluted with 3.0×10^{-3} mol/l NaOH. Working standard solution was obtained by appropriate dilution of the stock solution.

A 0.05 mol/l Tris–HCl buffer solution was prepared by dissolving of 3.03 g Tris in 500 ml volumetric flask with water and adjusted the pH to 9.75 with HCl.

All reagents were of analytical reagent grade without further purification, and doubly deionized water was used throughout.

2.3. General procedure

To a 25 ml test tube, working solutions were added in the following order: 1.0 ml of 1.0×10^{-4} mol/l Eu^{3+}; 1.0 ml of 1.0×10^{-3} mol/l OA; 1.0 ml of 0.05 mol/l Tris–HCl (pH 9.75); and appropriate amount of nucleic acid solutions. The mixture was diluted to 10 ml with doubly deionized water and shook to react efficiently and allowed to stand for 5 min. All RLS spectra were obtained by scanning simultaneously the excitation and emission monochromators (namely, $\Delta\lambda = 0$ nm) from 200 to 700 nm. The intensity of RLS was measured at $\lambda = 365$ nm in a 1 cm quartz cell with slit width at 10 nm for the excitation and emission. The enhanced RLS intensity of Eu^{3+}–OA system by nucleic acids was represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, here I_{RLS} and I^0_{RLS} were the intensities of the system with and without nucleic acids.

3. Results and discussion

3.1. RLS spectra and absorption spectra of Eu^{3+}–OA–DNA system

Fig. 1 shows the light scattering spectra of Eu^{3+}–OA–DNA system. Both OA and Eu^{3+}–OA show very weak RLS signals over the wavelength range of 200–700 nm. However, when Eu^{3+}–OA mixed with DNA in pH 9.75, the RLS intensity is strongly enhanced indicating an interaction between DNA and Eu^{3+}–OA. Fig. 2 is the absorption spectra of the system. It can be seen from line 1 that the absorption peak of OA located in 258, 266, 326 and 340 nm. Compare Fig. 1 with Fig. 2 according to the theory of RLS [2,14], the RLS peak at

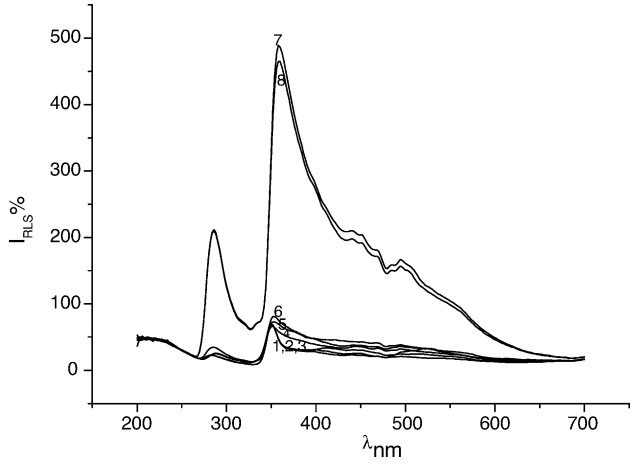

Fig. 1. Resonance light scattering spectra of the Eu^{3+}–OA–fsDNA system. (1) OA; (2) OA–Tris; (3) OA–Tris–fsDNA; (4) OA–Eu^{3+}; (5) Eu^{3+}–OA–fsDNA; (6) OA–Eu^{3+}–Tris; (7) OA–Eu^{3+}–Tris–fsDNA; (8) OA–Eu^{3+}–Tris–yRNA. Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH 9.75); fsDNA, 5.0×10^{-7} g/ml; yRNA, 5.0×10^{-7} g/ml.

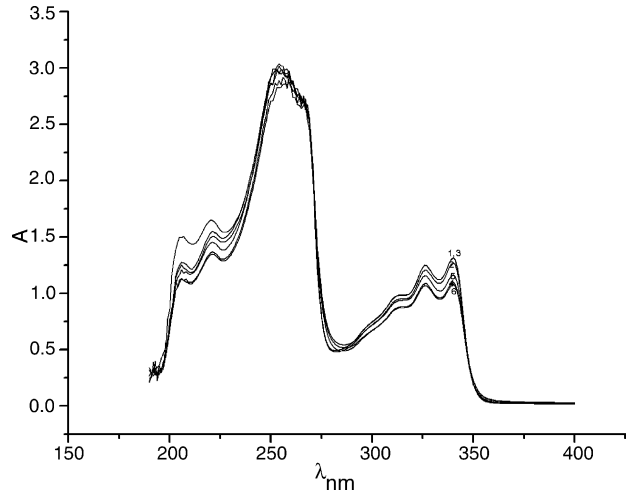

Fig. 2. Absorption spectra of Eu^{3+}–OA–fsDNA system. (1) OA; (2) OA–fsDNA; (3) OA–yRNA; (4) Eu^{3+}–OA; (5) Eu^{3+}–OA–fsDNA; (6) Eu^{3+}–OA–yRNA. Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml; fsDNA, 5.0×10^{-7} g/ml.

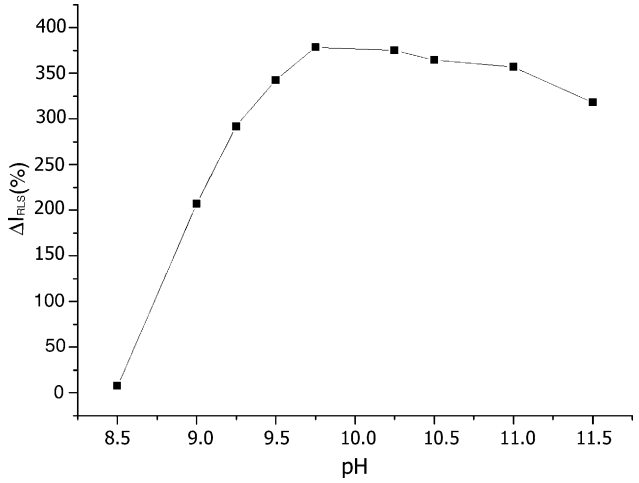

Fig. 3. Effect of pH on the RLS intensity. Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml; fsDNA, 5.0×10^{-7} g/ml.

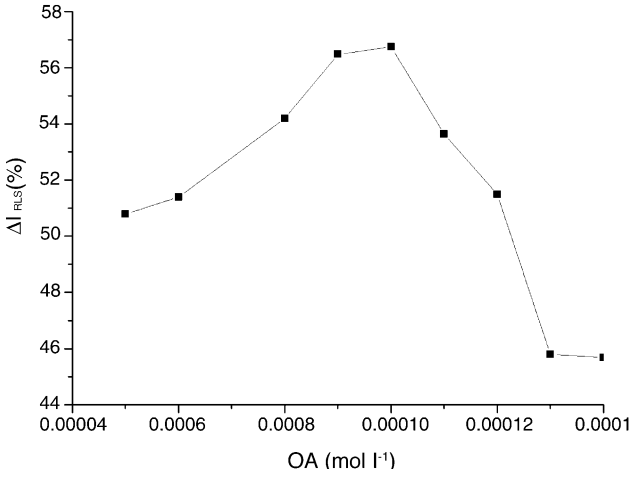

Fig. 4. Effect of the concentration of OA on the RLS intensity. Conditions: Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH 9.75); fsDNA, 5.0×10^{-7} g/ml.

300 and 368 nm comes from the cooperation of absorption of DNA and absorption of OA at 266 and 340 nm, respectively. In Fig. 1, the enhancement of RLS intensity of Eu^{3+}–OA by yRNA is also observed, and its pattern of the RLS spectra profiles is similar to that of Eu^{3+}–OA–fsDNA. However, the RLS intensity is little weaker than that of caused by fsDNA. Since RLS intensity at 368 nm is the maximum in both Eu^{3+}–OA–fsDNA and Eu^{3+}–OA–yRDNA systems, so 368 nm was selected in the further study.

3.2. Effect of pH and buffer solutions

Fig. 3 shows the relationship between the ΔI_{RLS} intensity and solution pH of the system. When pH is in the range from 9.5 to 10.2, the ΔI_{RLS} reaches the maximum and remain constant. Therefore, the change of pH had a great influence on ΔI_{RLS} intensity of the system. As shown in Fig. 3, pH 9.75 is the optimum condition for obtaining the maximum light scattering from Eu^{3+}–OA–DNA complex.

Under the condition of pH 9.7 ± 0.1, the effect of different kinds of buffers on ΔI_{RLS}(%) of the system was tested, and the ΔI_{RLS}(%) for Tris–HCl, NH_3–NH_4Cl, Na_2HPO_4–NaOH, HMTA, and $Na_2B_4O_7{\cdot}7H_2O$–NaOH buffer systems were 100, 36.1, 36.2, 73.8, and 33, respectively. The results show that the enhanced ΔI_{RLS} is the highest in Tris–HCl buffer solution and obtain maximum when the concentration of the Tris–HCl buffer is 0.05 mol/l. Therefore, 1.0 ml of 0.05 mol/l Tris–HCl buffer of pH 9.75 is selected for further study.

3.3. Effect of OA concentration

Effect of the OA concentration on the ΔI_{RLS}(%) was shown in Fig. 4. It can be seen that the maximum ΔI_{RLS}(%) obtained when concentration of OA is 1.0×10^{-4} mol/l. So in further experiments, the concentration of OA was fixed at 1.0×10^{-4} mol/l.

3.4. Effect of Eu^{3+} concentration

The effect of Eu^{3+} concentration on the ΔI_{RLS}(%) of the system was tested with OA concentration of 1.0×10^{-4} mol/l was shown in Fig. 5. It can be seen that the maximum ΔI_{RLS} intensity of the system occurs at 1.0×10^{-5} mol/l Eu^{3+}.

3.5. Effect of addition order

The effect of addition order on the ΔI_{RLS}(%) of the system was tested. Result shows that the order of Eu^{3+}–OA–Tris–DNA is the best.

3.6. Incubation time and the stability of the system

The influence of incubation time on the RLS enhanced by fsDNA was investigated. The experiment shows that the

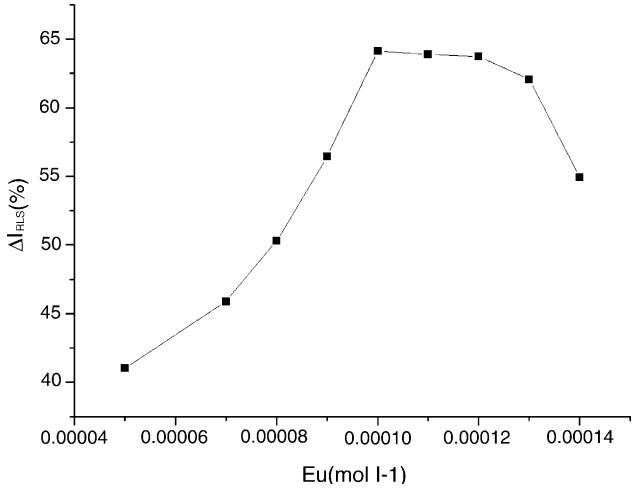

Fig. 5. Effect of the concentration of Eu^{3+} on the RLS intensity. Conditions: OA, 1.0×10^{-4}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH 9.75); fsDNA, 5.0×10^{-7} g/ml.

Table 1
Effects of foreign substances on the RLS intensities

Foreign substances (mol/l)	ΔI_{RLS} (%)
BSA, 4.6×10^{-6} (g/ml)	+4.9
Val, 4.0×10^{-4}	+5.0
HSA, 2.0×10^{-6} (g/ml)	+7.6
L-Phe, 3.0×10^{-4}	−4.3
Na^+, Cl^-, 2.5×10^{-4}	−5.2
L-Asp, 4.5×10^{-4}	−4.4
K^+, Cl^-, 1.2×10^{-4}	−4.8
Cys, 1.0×10^{-5}	+4.7
Ba^{2+}, Cl^-, 3.0×10^{-5}	−5.1
Uracil, 5.0×10^{-6}	+5.0
Al^{3+}, Cl^-, 2.0×10^{-6}	+4.6
Thymine, 1.03×10^{-4}	−4.8
Fe^{3+}, Cl^-, 8.0×10^{-7}	+5.1
L-His, 4.0×10^{-4}	−2.0
Mg^{2+}, SO_4^{2-}, 6.4×10^{-6}	−4.8
DL-Thr, 3.0×10^{-4}	−3.4
Zn^{2+}, SO_4^{2-}, 4.4×10^{-5}	−5.0
L-Ala, 3.0×10^{-4}	−2.6
Gd^{3+}, 1.78×10^{-6}	+4.6
AMP, 1.8×10^{-6}	+4.3
Y^{3+}, 2.5×10^{-6}	+4.8
Tb^{3+}, 2.0×10^{-6}	+4.8

OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH 9.75); fsDNA, 5.0×10^{-7} g/ml.

ΔI_{RLS} intensity reaches the maximum within 5 min after the mixing of the solutions and remains stable for at least 24 h, which means that the system is very stable for the determination of nucleic acids.

3.7. Interference study

The influence of various ions, proteins, nucleotides and other microbiomoleculars was tested at pH 9.75 when the concentration of fsDNA was 5.0×10^{-7} g/ml. The results are listed in Table 1. As we can see, most of them have little interference with the determination of nucleic acids, so good selectivity is found for this method.

3.8. Analytical applications

Under optimal conditions, linear relationships between the enhanced RLS (ΔI_{RLS}) and the nucleic acid concentrations (c) are constructed. All the analytical parameters have been regressed, and are presented in Table 2. It demonstrated that the linear relationship between ΔI_{RLS} and the concentration of nucleic acids over a wide range. The limits of detection all reached 10^{-11} g/ml. A comparison of this method with other well-known methods, in terms of sensitivity is summarized in Table 3. It can be seen that the sensitivity of this method is much higher than most other methods. Therefore, this method is rapid, simple and sensitive, and will be a valuable tool for studying the biological properties of nucleic acids.

Table 2
Analytical parameters for the determination of nucleic acids

Nucleic acids	Linear range (g/ml)	r[a]	LOD[b] (ng/ml)
fsDNA	1.0×10^{-9} to 1.0×10^{-6}	0.9959	0.020
ctDNA	8.0×10^{-8} to 1.0×10^{-6}	0.9932	0.011
yRNA	1.0×10^{-9} to 1.0×10^{-6}	0.9929	0.010

Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH = 9.75).
[a] Correlation coefficient.
[b] Limit of detection (S/N = 3).

The present method was applied to determine nucleic acids in synthetic samples constructed based on the tolerance of co-existing species: 1.0×10^{-7} g/ml, BSA; 1.0×10^{-5} mol/l, thymine; 2.0×10^{-5} mol/l, Val; 3.0×10^{-6} mol/l, $ZnCl_2$; 5.0×10^{-6} mol/l, KCl. The recovery of the synthetic sample is 96.5–104.1%, and the results obtained were satisfactory. The proposed method was also used to determine the DNA in an actual sample, which was isolated from common wheat (*Triticum aestivum* L.) derived from granular embryogenic calli according to the CTAB method [26]. The results, which obtained by this method and the UV method, were shown in Table 4. It can be seen that this method is reliable, precise and simple.

3.9. The mechanism of interaction of Eu^{3+}–OA–DNA system

The fluorescence spectra of the system were shown in Fig. 6. It can be seen that OA can remarkably enhance the fluorescence intensity of Eu^{3+} in aqueous solution, which indicated that there was strong interaction between Eu^{3+} and OA. It also showed that fsDNA can remarkably quench the fluorescence intensity of Eu^{3+}–OA complex. From above phenomenon, we predict that interaction between Eu^{3+}–OA and fsDNA caused the enhancement of RLS intensity of Eu^{3+}–OA. From absorption spectra, we can see that the addition of fsDNA only lead to a slightly increasing of the absorption of Eu^{3+}–OA, without the phenomenon of absorp-

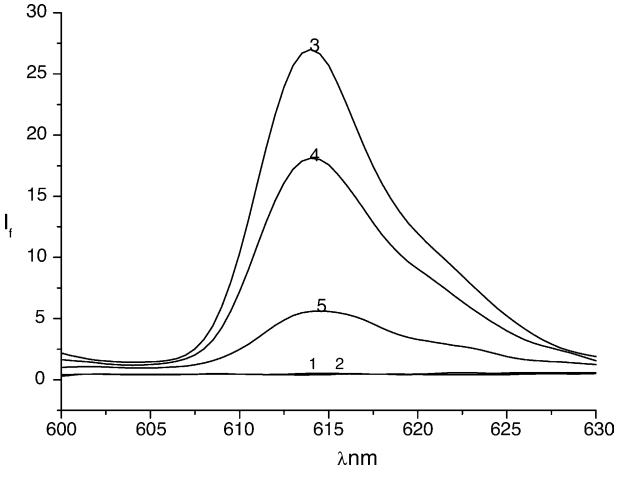

Fig. 6. Fluorescence spectra of the system. (1) Eu^{3+}; (2) Eu^{3+}–fsDNA; (3) Eu^{3+}–OA; (4 and 5) Eu^{3+}–OA–fsDNA. Conditions: OA, 1.0×10^{-4} mol/l; Eu^{3+}, 1.0×10^{-5}, 0.05 mol/l; Tris–HCl, 1.0 ml (pH 9.75); fsDNA, 1.0×10^{-6} g/ml, 3.0×10^{-6} g/ml.

Table 3
Common probes for nucleic acids

Probes	Method	Nucleic acids	LOD[a] (ng/ml)	References
Hoechst 33258	FL[b]	DNA	10	[15]
Tb^{3+}-phenanthroline	FL	ctDNA/fsDNA/yRNA	100/200/200	[16]
Pico green	FL	dsDNA[c]	0.025	[17]
Phosphin 3R	FL	ctDNA/smDNA/yRNA	5.0/6.0/13.0	[18]
$Ru(bipy)_2(dppx)^{2+}$	FL	ctDNA/ssDNA/fsDNA	0.75/0.66/1.49	[19]
Ethdium bromide	FL	DNA	10	[20]
Azur A	RLS	ctDNA/fsDNA	19.9/12.6	[21]
TAAlPc[d]	RLS	ctDNA/fsDNA/yRNA	1.4/1.4/2.7	[22]
Acridine Red	RLS	ctDNA/fsDNA/yRNA	0.095/1.3/8.5	[23]
Morin-CTMAB	RLS	ctDNA/fsDNA/yRNA	3.4/6.2/4.1	[24]
Berberine	RLS	fsDNA/ctDNA/yRNA	6.5/2.1/3.5	[25]
TAPP[e]	RLS	ctDNA/fsDNA/yRNA	4.1/4.6/6.7[f]	[4]
This probe	RLS	fsDNA/ctDNA/yRNA	0.020/0.011/0.010	

[a] Limits of detection.
[b] Fluorimetric method.
[c] Double strand DNA.
[d] Tetra-substituted amino aluminum phthalocyanine.
[e] α,β,γ,δ-tetrakis[4-(trimethylammoniumyl)phenylporphine.
[f] $\times 10^{-8}$ mol/l.

Table 4
The determination of DNA actual sample

Sample	Wheat DNA found ($\times 10^{-7}$ g/ml)		Average ($\times 10^{-7}$ g/ml)	R.S.D. (%)
	UV method	This method		
1	3.75	3.73, 3.78, 3.70, 3.72, 3.76	3.74	0.33

tion reducing and red shift of peak. So we consider that Eu^{3+}–OA has not insert into the base sequence of DNA. As we known, DNA possessed negative charges due to the phosphate group, so we consider that there was strong electrostatic interaction between DNA and Eu^{3+}–OA, and Eu^{3+} as a bridge combined fsDNA with OA to form a complex, which lead to the significant increasing of I_{RLS}. The influence of PO_4^{3-} on the system also demonstrated the electrostatic action between DNA and Eu^{3+}–OA as shown in Fig. 7. It can be seen that

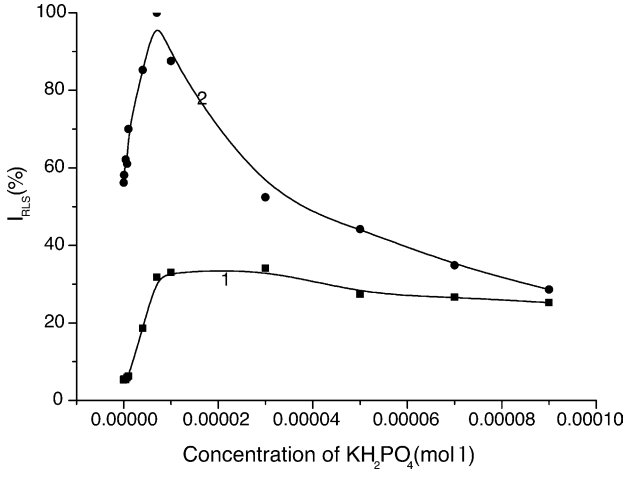

Fig. 7. Effect of the concentration of PO_4^{3-} on the RLS intensity. (1) Eu–OA–Tris–HCl; (2) Eu–OA–fsDNA–Tris–HCl. Conditions: OA, 1.0×10^{-4} mol/l; Eu, 1.0×10^{-5} mol/l; Tris–HCl, 1.0 ml (pH 9.75), 0.05 mol/l; fsDNA, 5.0×10^{-7} g/ml.

the I_{RLS} of both the Eu^{3+}–OA and Eu^{3+}–OA–DNA systems are increased when the concentration of PO_4^{3-} is low and reached maximum at 7.0×10^{-6} mol/l PO_4^{3-}. Then the I_{RLS} of Eu^{3+}–OA–DNA system reduced with the increasing of concentration of PO_4^{3-}, while the I_{RLS} of Eu^{3+}–OA system tend to changeless. We consider that a little bit of PO_4^{3-} can neutralize the Eu^{3+} whose coordinate number is not saturated, which contribute to the broadening of the complex across area that lead to the increase of I_{RLS}. But excessive PO_4^{3-} will compete with DNA for combining with Eu^{3+}, so the I_{RLS} of Eu^{3+}–OA–DNA system was decreased. Figs. 8 and 9 are the transmission electronic microscope (TEM) pictures of fsDNA and Eu^{3+}–OA–DNA, respectively. From the pictures

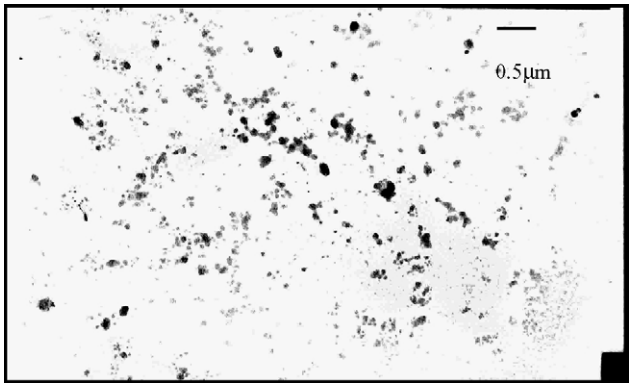

Fig. 8. TEM of fsDNA at pH 9.75.

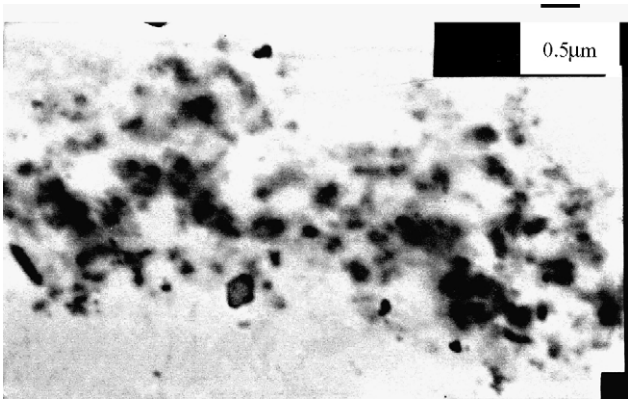

Fig. 9. TEM of Eu^{3+}–OA–DNA at pH 9.75. Conditions: OA, 1.0×10^{-4} mol/l; Eu, 1.0×10^{-5} mol/l; Tris–HCl, 1.0 ml (pH 9.75), 0.05 mol/l; fsDNA, 5.0×10^{-7} g/ml.

we can see that under the same magnifying power the shape of fsDNA (pH 9.75) is dispersed particles, while after the addition of Eu^{3+} and OA, the particles are congregate into agglomerates, and the radius are obvious larger. From this fact we conclude that the increase of the radius of scattering particles contribute to the enhancement of RLS intensity.

4. Conclusion

The interaction of Eu^{3+}–OA with nucleic acids is investigated using RLS technique. Experiments showed that Eu^{3+}–OA cooperate with DNA by electrostatic. Thereby, a sensitive and convenient method for the determination of nucleic acids is established. The limit of detection reaches 10^{-11} g/ml. Besides that, the Eu^{3+}–OA–DNA system can tolerate most interfering substances, including proteins, nucleotides and metal ions. The results suggest that Eu^{3+}–OA complex may be developed as a new promising kind of probes for the determination of trace amount of nucleic acids.

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