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# Fluorometric Determination of Nucleic Acids Using A Fluorescence-Enhancing Method

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It was found that the fluorescence intensity of gadolinium-quercetin system was greatly enhanced by nucleic acids in the presence of sodium dodecyl benzene sulfonate. Based on this, nucleic acids can be sensitively determined by the fluorescence technique. The enhanced fluorescence intensity was proportional to the concentration of nucleic acids among a wide range. The detection limits ranged from 5.0 to 21 ng/mL depending on the kind of nucleic acids. The interaction mechanism between gadolinium-quercetin-sodium dodecyl benzene sulfonate and nucleic acids was electrostatic binding.

**Key Words: Fluorescence, Nucleic acids, Quercetin.**

## INTRODUCTION

Nucleic acids are important biologic giant molecule and the carrier of genetic information. They are also the pivotal factor for species' continuation and evolution. The quantitative analysis of nucleic acids is important for the disease diagnoses and the test of therapy effects and is the common analytical item in the food examination and quality test of many biochemical medicines' separation and purification and has intrigued biochemistry and other biological subjects greatly. But the direct determination of nucleic acids by the intrinsic fluorescence and ultraviolet absorption is severely limited by low sensitivity[1-5]. Some method based on quenching or enhancing the fluorescence of various components after the interaction with nucleic acid which named probe technique was proposed[6,7]. Probe technique is the important means to studying structure, function, qualitative and quantitative analyses of nucleic acids. Some probes such as organic dye[8-10], fluorescent complex[11-16] and lanthanide metal ion[17-19] have been used to improve the sensitivity and selectivity for nucleic acid determination. In present paper, the luminescence probe of gadolinium(III) ($Gd^{3+}$)-quercetin (Qu)-sodium dodecyl benzene sulfonate (SDBS) is advanced which has convenient, rapid and sensitive advantage for the determination of nucleic acids.

## EXPERIMENTAL

Stock solutions of nucleic acids ($1.0 \times 10^{-4}$ g/mL) were prepared by dissolving 0.0100 g commercial herring sperm DNA (fsDNA, Sigma), calf thymus DNA (ctDNA, Sigma) and yeast RNA (yRNA, Sinopharm Chemical Reagent) in 100 mL water. Stock solution of $Gd^{3+}$ ($1.0 \times 10^{-2}$ mol/L) was prepared by dissolving 0.1629 g

gadolinium oxide (99.99 %, Shanghai Maikun Chemical) with 10 mL hydrochloric acid and then diluting to 100 mL with water. Stock solution of quercetin ($1.00 \times 10^{-3}$ mol/L) was prepared by dissolving 0.0302 g of quercetin in100 mL ethanol. Stock solution of sodium dodecyl benzene sulfonate (SDBS) ($1.00 \times 10^{-2}$ mol/L) was prepared by dissolving 0.8712 g of SDBS (Sinopharm Chemical Reagent) in 250 mL water. Stock solution of hexamethylenetetramine (HMTA, 4 %) was prepared by dissolving 8.00 g HMTA in 200 mL water.

All the chemicals used were analytical grade. Water used throughout was doubly deionized distilled water.

The fluorescence spectra were recorded with a LS-55 fluorescence spectrometer (Perkin-Elmer). The absorption spectra were recorded with a UV-2450 spectrophotometer (Shimadzu). All pH measurements were made with a PHSJ-4A laboratory pH meter (Leici, Shanghai). The surface tension was measured on processor tensiometer-K12 (Krüss Corp.).

**Procedure:** The required amount of solutions was successively added in the following order: HMTA, $Gd^{3+}$, Qu, SDBS and nucleic acids. The mixture was diluted with water and mixed thoroughly, then measured after 20 min. The excitation and emission peaks were at 447 and 512 nm, respectively. The enhanced fluorescence intensity of $Gd^{3+}$-Qu-SDBS-nucleic acids system was represented as $\Delta I_f = I_f - I_f^0$. Here $I_f$ and $I_f^0$ were the fluorescence intensity with and without nucleic acids.

## RESULTS AND DISCUSSION

**Excitation and emission spectra:** Excitation and emission spectra of Qu, $Gd^{3+}$-Qu-SDBS, $Gd^{3+}$-Qu-SDBS-ctDNA, $Gd^{3+}$-Qu-SDBS-yRNA and $Gd^{3+}$-Qu-SDBS-fsDNA systems were shown in Fig. 1 which indicated that with the excitation wavelength of 447 nm, the $Gd^{3+}$-Qu-SDBS system emitted the characteristic fluorescence of Qu with the emission peak of 512 nm and the fluorescence intensity can be greatly enhanced by nucleic acids.

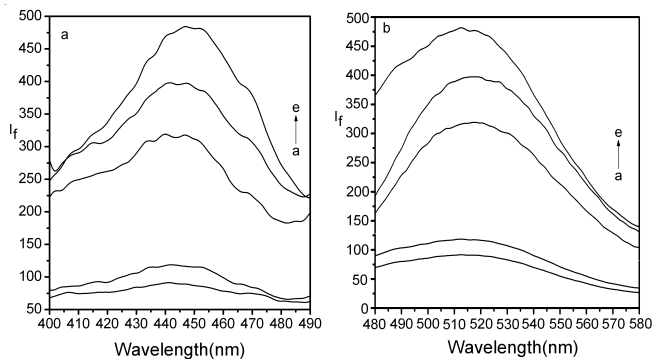


Fig. 1. Excitation (a) and emission (b) spectra a: Qu; b: $Gd^{3+}$-Qu-SDBS; c: $Gd^{3+}$-Qu-SDBS-ctDNA; d: $Gd^{3+}$-Qu-SDBS-yRNA; e: $Gd^{3+}$-Qu- SDBS-fsDNA; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; fsDNA: $5.0 \times 10^{-6}$ g/mL; ctDNA: $5.0 \times 10^{-6}$ g/mL; yRNA: $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25

**Effect of Buffer solution and pH value:** Effect of pH value on the fluorescence intensity was shown in Fig. 2 which indicated that the maximum ΔIf was obtained at pH value 5.25.

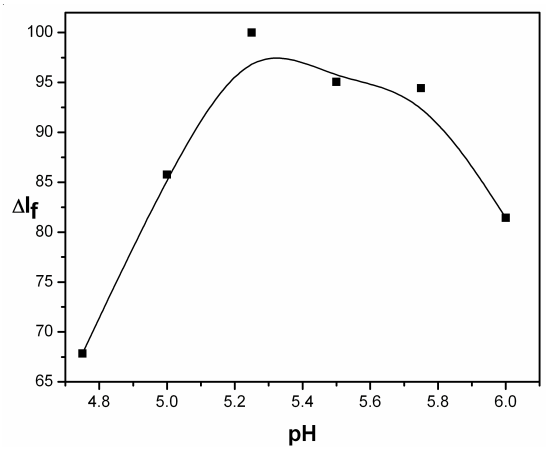


Fig. 2. Effect of pH value Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %

The $\Delta I_f$ for HMTA-HCl, $NH_4Cl$-$NH_3$, *tris*-HCl, $Na_2B_4O_7$-$H_2BO_3$ and $NaH_2PO_4$-$Na_2HPO_4$ was 100, 4.5, -0.2, -1.7 and -4.2, respectively, which showed that HMTA-HCl was the most suitable buffer solution. Further studies demonstrated the optimal concentration of HMTA was 0.8 %.

**Effect of quercetin (Qu) and $Gd^{3+}$:** Figs. 3 and 4 showed that the optimal concentration of quercetin and $Gd^{3+}$ was $5.0 \times 10^{-6}$ mol/L and $1.0 \times 10^{-4}$ mol/L, respectively.

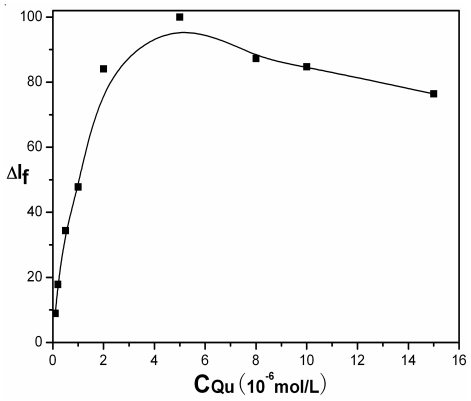


Fig. 3. Effect of Qu; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; fsDNA: $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25

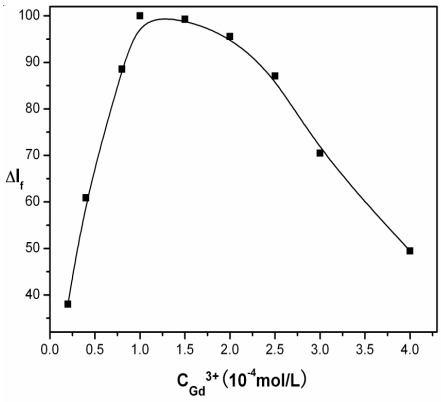


Fig. 4. Effect of $Gd^{3+}$; Conditions: Qu: $5.0 \times 10^{-6}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; fsDNA: $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25

**Effect of sodium dodecyl benzene sulfonate (SDBS):** Different surfactants had different influence on the fluorescence intensity of the system. The $\Delta I_f$ for SDBS, TX-100, betaine, SLS and CTMAB was 100, 99.0, 93.77, 93.02 and 2.0, which indicated that SDBS was the most suitable surfactant. Fig. 5 showed that the optimal concentration of SDBS was $2.0 \times 10^{-4}$ mol/L.

Fig. 6 showed that the critical micelle concentration (CMC) of SDBS in this system was $1.5 \times 10^{-4}$ mol/L, while the optimum SDBS concentration ($2.0 \times 10^{-4}$ mol/L) in this experiment was larger than its CMC. So, SDBS existed as micelle.

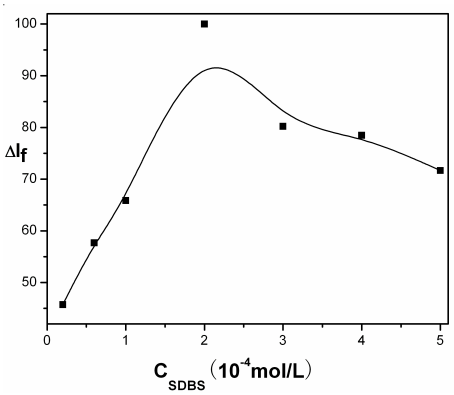


Fig. 5. Effect of SDBS; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; fsDNA: $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25

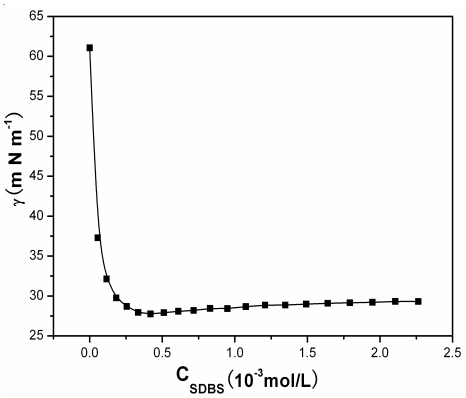


Fig. 6. Surface tension of SDBS; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; fsDNA: $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25

**Effect of addition order and time evolution:** Effect of addition order was tested and results indicated that the optimum addition order was HMTA, $Gd^{3+}$, Qu, SDBS and nucleic acids.

Effect of time evolution on the fluorescence intensity was studied and the results showed that $\Delta I_f$ reached a maximum after 20 min and remained stable for over 10 h. Therefore, this system exhibited good stability.

**Effect of foreign substances:** Interference of foreign substances was tested and shown in Table-1. It was found that most of ions had little effect on the determination of $1.0 \times 10^{-6}$ g/mL fsDNA within the permissible ± 5 % error.

## Analytical applications

**Calibration graphs and detection limits:** Under the optimum condition defined, the calibration graphs for nucleic acids determination were obtained and shown in Table-2. It can be seen that there was a linear relationship between the $\Delta I_f$ and the concentration of nucleic acids which ranged from $2.0 \times 10^{-8}$ to $3 \times 10^{-5}$ g/mL for fsDNA, from $5.0 \times 10^{-8}$ to $3 \times 10^{-5}$ g/mL for yRNA, from $5.0 \times 10^{-8}$ to $3 \times 10^{-5}$ g/mL for ctDNA. Their detection limits (S/N = 3) were 5.0, 20.0 and 21.0 ng/mL, respectively.

TABLE-1
EFFECT OF FOREIGN SUBSTANCES

| Substances | Concentration coexisting ($\times10^{-5}$ mol/L) | Change of $\Delta I_f$ (%) | Substances | Concentration coexisting ($\times10^{-5}$ mol/L) | Change of $\Delta I_f$ (%) |
|---|---|---|---|---|---|
| $Zn^{2+}$, $Cl^-$ | 8 | -1.5 | DL-threonine | 2 | +4.0 |
| $Fe^{3+}$, $Cl^-$ | 1 | -4.0 | L-histidine | 10 | -2.8 |
| $K^+$, $Cl^-$ | 2 | -5.0 | Tryptophan | 2 | -4.1 |
| $Ca^{2+}$, $Cl^-$ | 8 | -1.7 | L-Asp [a] | 32 | -4.1 |
| $Mg^{2+}$, $Cl^-$ | 4 | +4.0 | DL-Glu [b] | 36 | -4.0 |
| $Mn^{2+}$, $Cl^-$ | 12 | +4.0 | UTP [c] | 2 | -3.8 |
| $Ba^{2+}$, $Cl^-$ | 69 | +5.0 | AMP [d] | 9 | +4.2 |
| $NH_4^+$, $Cl^-$ | 50 | -3.5 | GMP [e] | 8 | -3.6 |
| $Na^+$, $Cl^-$ | 60 | +2.6 | BSA [f] | $6\times10^{-4}$g/mL | +4.5 |
| Cysteine | 8 | +3.0 | HSA [g] | $2\times10^{-4}$g/mL | -3.6 |
| Tyrosine | 6 | -3.2 | EA [h] | $1\times10^{-4}$g/mL | +4.0 |

a: L-Aspartic acid; b: Glutamic acid; c: Uridine-5'-triphosphate; d: Adenosine monophosphate; e: Guanosine monophosphate; f: bovine serum albumin; g: human serum albumin; h: egg albumin; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; fsDNA: $1.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25.

TABLE-2
ANALYTICAL PARAMETERS

| DNA | Linear range (g/mL) | Linear regression equation (g/mL) | Correlation coefficient | Detection limit (ng/mL) |
|---|---|---|---|---|
| fsDNA | $2.0\times10^{-8}$-$3\times10^{-5}$ | $\Delta I_f = 2.21\times10^7C + 9.87$ | 0.996 | 5.0 |
| yRNA | $5.0\times10^{-8}$-$3\times10^{-5}$ | $\Delta I_f = 6.32\times10^6C + 18.99$ | 0.992 | 20.0 |
| ctDNA | $5.0\times10^{-8}$-$3\times10^{-5}$ | $\Delta I_f = 6.48\times10^6C + 14.38$ | 0.996 | 21.0 |

Conditions: $Gd^{3+}$: $1.0\times10^{-4}$ mol/L; Qu: $5.0\times10^{-6}$ mol/L; SDBS: $2.0\times10^{-4}$ mol/L; HMTA: 0.8%; pH = 5.25.

**Determination of actual samples:** The standard addition method was used for the determination of yRNA. The yRNA actual sample was made from yeast solution using an alkaline method[20] and analyzed using this method and compared with UV spectrophotometric method. The results (Table-3) showed that the accuracy and precision of the method were satisfactory.

TABLE-3
ANALYSIS OF yRNA SAMPLES

| Sample | Methods | Concentration (mg/mL) | Average (mg/mL) | RSD (%) |
|---|---|---|---|---|
| yRNA | Proposed method | 55.9, 56.2, 55.5, 56.2, 55.8 | 55.85 | 0.29 |
| | UV method | 56.6, 57.2, 56.4, 57.4, 56.9 | 56.90 | 0.41 |

Conditions: $Gd^{3+}$: $1.0\times10^{-4}$ mol/L; Qu: $5.0\times10^{-6}$ mol/L; SDBS: $2.0\times10^{-4}$ mol/L; g/mL; HMTA: 0.8%; pH=5.25

**Interaction mechanisms:** Fig. 1 showed that the $Gd^{3+}$-Qu-SDBS-nucleic acids system had the excitation band of 320-380 nm. From Fig. 7, it was found that quercetin had obvious absorption, whereas SDBS and nucleic acids had not among 320-380 nm. The facts indicated the fluorescence of $Gd^{3+}$-Qu-SDBS-nucleic acids system was from the absorption of quercetin rather than SDBS and nucleic acids.

Fig.1 showed that the fluorescence enhancement of single helix yRNA was greater than that of double helix fsDNA, but lower than that of double helix ctDNA, which indicated that the interaction mechanism between $Gd^{3+}$-Qu-SDBS and nucleic acids was in the mode of electrostatic binding rather than intercalation or groove binding.

The variety of fluorescence polarization value is one of the standards to estimate the binding mode between small molecules and nucleic acids. After small molecules inset the bases, the running of small molecules is suffocated which results in the increasing of fluorescence polarization. Fig. 8 showed the fluorescence polarization value was reduced after the adding of fsDNA, which indicate the interaction mechanism between $Gd^{3+}$-Qu-SDBS and nucleic acids was not intercalation binding.

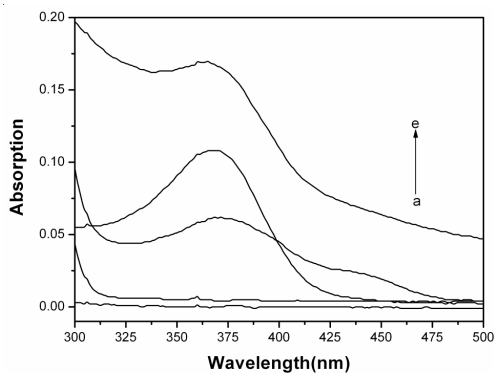


Fig. 7. Absorption spectra, a: SDBS; b: fsDNA; c: Qu-$Gd^{3+}$-SDBS; d: Qu; e: Qu-$Gd^{3+}$-SDBS-DNA; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; fsDNA: $5.0 \times 10^{-6}$ g/mL; HMTA: 0.8 %; pH = 5.25

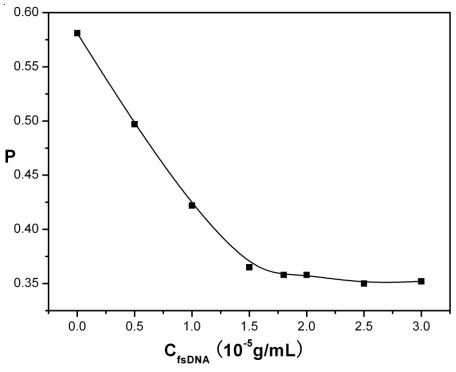


Fig. 8. Fluorescence polarization value; Conditions: $Gd^{3+}$: $1.0 \times 10^{-4}$ mol/L; Qu: $5.0 \times 10^{-6}$ mol/L; SDBS: $2.0 \times 10^{-4}$ mol/L; HMTA: 0.8 %; pH = 5.25

## Conclusion

In this paper, a new fluorimetric method of determination of nucleic acids was developed. Under optimum conditions, the enhanced intensity of fluorescence was in proportional to the concentration of nucleic acids among wide range. The interaction mechanism between $Gd^{3+}$-Qu-SDBS and nucleic acids was the mode of electrostatic binding.

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