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Resonance light scattering study on the interaction between quinidine sulfate and congo red and its analytical application

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ABSTRACT: The interaction between quinidine sulfate (QDS) and congo red (CR) was studied using resonance light scattering (RLS) technique, ultraviolet–visual spectrophotometry and fluorimetry. In weak acidic medium, QDS reacts with CR to form a supermolecular complex which results in the enhanced RLS intensity. Some important interacting parameters, such as the solution acidity and CR concentration, salt effect and addition order of the reagents, were investigated and optimized. Under the optimum conditions, it was found that the enhanced RLS intensity was in proportion to the concentration of QDS in the range 0.2–8.4 $\mu\text{g mL}^{-1}$. The corresponding detection limit was 12.0 ng mL^{-1} . The results showed that this new method enabled simple, sensitive and rapid determination of QDS and was used for the determination of QDS in urine and simulated huamn serum samples. Copyright © 2009 John Wiley & Sons, Ltd.

Keywords: resonance light scattering; quinidine sulfate; congo red

Introduction

Quinidine sulfate (QDS) is an antiarrhythmic drug which can be used to suppress ventricular fibrillation and prevent antitachycardia pacing.^[1–3] However, when patients were administered doses of quinidine sulfate sufficient to produce serum concentrations of higher than 2.5 $\mu\text{g mL}^{-1}$, it resulted in side effects such as drug allergy, exacerbation of weakness and the development of respiratory failure.^[4] Therefore, to take full advantage of the antiarrhythmic drug and to decrease its toxicity, it is essential to quantitatively determine QDS in clinical samples.

Several methods have been used for the determination of QDS (the molecular structure of QDS is shown in Fig. 1), including fluorimetry (FLU),^[5] high-performance liquid chromatography (HPLC),^[6] capillary electrophoresis (CE)^[7] and fluorescence microscopic analysis (FMA).^[8] FLU exhibits good sensitivity for QDS quantification; however, the narrow linear range limits its application range.^[5] HPLC and CE have relatively good sensitivity, accuracy and precision, especially low disturbance, but the procedure is complicated and time-consuming.^[6,7] FMA has been used owing to its high sensitivity and small sample consumption, but this method is easily affected by large amounts of surfactants and salts.^[9]

Analytical procedures applying the RLS method combine the advantages of simplicity, sensitivity and rapidity.^[10–14] Since Huang *et al.* first used this technique for analytical purposes,^[10]

much attention has been paid to the study and determination of nucleic acids,^[15–17] proteins^[18–20] and inorganic ions^[21–23] by use of this method. In recent years, RLS has been widely applied to determine some pharmaceuticals.^[24–26] Until now, there has been no report concerning the determination of QDS using RLS. The aim of this work is to develop a simple, sensitive and rapid method for the determination of QDS that gives results comparable to those obtained by the existing methods.

Congo red, a kind of bisazo anionic dyes, is a sensitive RLS probe and has been used to determine many chemicals, such as proteins,^[27] nucleic acids^[28] and drugs.^[29–31] In the experiments of this paper, the interaction between QDS and CR to form a supermolecular complex was studied using RLS. The faint RLS intensity of congo red was greatly enhanced by the addition of QDS. Under the optimum experimental conditions, the RLS intensity change was linear with the concentration of QDS in the range 0.2–8.4 $\mu\text{g mL}^{-1}$ with a detection limit of 12.0 ng mL^{-1} . The proposed method exhibited high precision and was successfully applied to the determination of QDS in urine and simulated huamn serum samples.

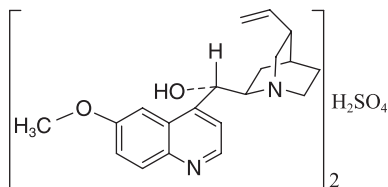


Figure 1. The molecular structure of QDS.

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Experimental

Apparatus

The RLS and fluorescence spectra were measured with a Cary Eclipse fluorescence spectrophotometer (Varian, USA) with a quartz cuvette (1 × 1 cm). A UV2550 spectrophotometer (Shimadzu, Japan) was used for measuring absorption spectra. A Delta 320 pH meter (Mettler-toledo instrument corporation, Shanghai, China) was used for pH measurement.

Reagents

A stock solution of 1 g L⁻¹ quinidine sulfate was prepared by dissolving 0.105 g quinidine sulfate dihydrate (Sigma, USA) in 100 mL water. The concentration of the working solution was made up to 1.0 × 10⁻³ mol L⁻¹ by diluting the QDS stock solution with water. The stock and working solutions were stored in dark environment.

The stock solution of congo red (analytical-reagent grade, Shanghai Chemistry Reagent Corporation, China) was made up to 1.0 × 10⁻³ mol L⁻¹ by dissolving CR in water. The working solution was prepared by diluting the stock solution to 1.0 × 10⁻⁴ mol L⁻¹ with water.

Britton Robinson buffer solutions with different pH (pH 2.5–6.0) were prepared by mixing the mixed acid (composed of 0.04 mol L⁻¹ H₃PO₄, HAc and H₃BO₃) with 0.2 mol L⁻¹ NaOH in proportion. The buffer was used to control the acidity of the interacting system.

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

Sample preparation

Human serum and urine samples were provided by a local hospital. To prepare serum samples, a 1.0 mL aliquot of serum sample and 0.25 mL trichloroacetic acid were mixed thoroughly and centrifuged at 4000 rpm for 10 min. A 0.5 mL aliquot of the supernatant fluid was diluted to 1000-fold with water without further purification. Then QDS in serum samples was determined according to the general procedure. Urine samples were directly determined according to the general procedure without simple treatment.

The compositions of simulated serum samples (SMSS) were as follows: 5.14 μg mL⁻¹ methionine, 5.95 μg mL⁻¹ cysteine, 14.3 μg mL⁻¹ tryptophan, 1.52 μg mL⁻¹ tyrosine, 1.84 μg mL⁻¹ histidine, 12.6 μg mL⁻¹ serine, 11.2 μg mL⁻¹ glycine, 26.4 μg mL⁻¹ phenylalanine, 29.2 μg mL⁻¹ lysine, 38.3 μg mL⁻¹ arginine, 36.5 μg mL⁻¹ alanine, 119.7 μg mL⁻¹ aspartic acid, 0.84 g L⁻¹ NaHCO₃ and 3.85 g L⁻¹ NaCl. The simulated serum samples were diluted 10-fold for the determination and recovery tests.

General procedure

A 1.0 mL aliquot of Britton–Robinson buffer, 1.0 mL CR working solution and appropriate QDS (or samples) were added to a 10 mL calibrated flask. The resulting solution was diluted to the mark with water and then mixed thoroughly.

All RLS spectra were obtained by simultaneously scanning the excitation and emission monochromators ($\Delta\lambda = 0.0$ nm) from 230.0 to 750.0 nm with the excitation and emission slits 5.0 nm. The RLS intensities were measured at 562 nm. The enhanced RLS

intensity of the system was represented as $\Delta I_{\text{RLS}} = I_{\text{RLS}} - I_0$ (I_{RLS} and I_0 are the intensities of the systems with and without QDS).

Results and discussion

RLS spectra

Figure 2 shows the RLS spectra of CR, QDS and QDS-CR system at pH 4.5. It can be seen from Fig. 2 that RLS intensities of the CR and QDS are weak in the wavelength range of 230–750 nm. However, the enhanced RLS intensity can be clearly observed with three peaks located at 272, 388 and 562 nm when a trace amount of QDS was added to the solution of CR. Moreover, the enhanced RLS intensity increased with the increasing QDS concentration. The maximum RLS peak was located at 562 nm. Therefore, 562 nm was selected as the analytical wavelength.

Effect of acidity

As shown in Fig. 3, the effect of the solution acidity on the scattering intensity of the system was investigated. Variation of pH from 2.5 to 6.0 at the concentration of 6 μg mL⁻¹ of QDS was studied. At pH 4.5, the enhanced RLS intensity reached its

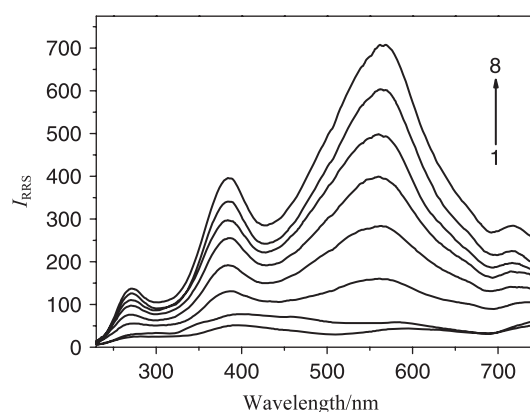


Figure 2. RLS spectra of the QDS-CR system. Conditions—(1) C_{QDS} 6.0 μg mL⁻¹, pH 4.5; (2) C_{CR} 1.0 × 10⁻⁵ mol L⁻¹, pH 4.5; (3–8) QDS + CR, C_{CR} 1.0 × 10⁻⁵ mol L⁻¹, C_{QDS} (3–8, μg mL⁻¹): (3) 2.0; (4) 3.0; (5) 4.0; (6) 5.0; (7) 6.0; (8) 7.0, pH 4.5.

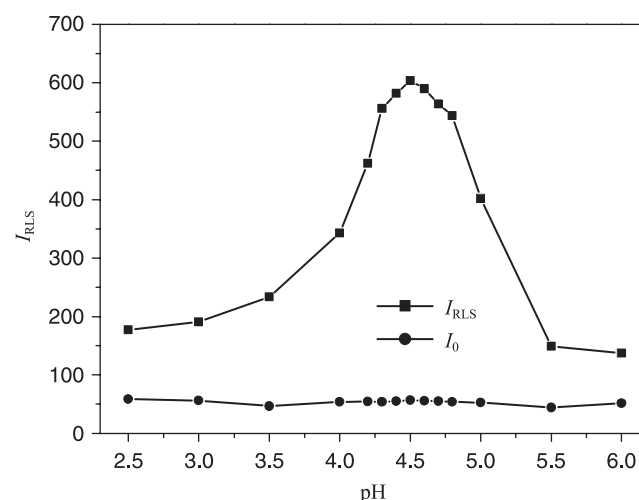


Figure 3. Effect of pH on the RLS intensity. Conditions— C_{QDS} 6.0 μg mL⁻¹, C_{CR} 1.0 × 10⁻⁵ mol L⁻¹.

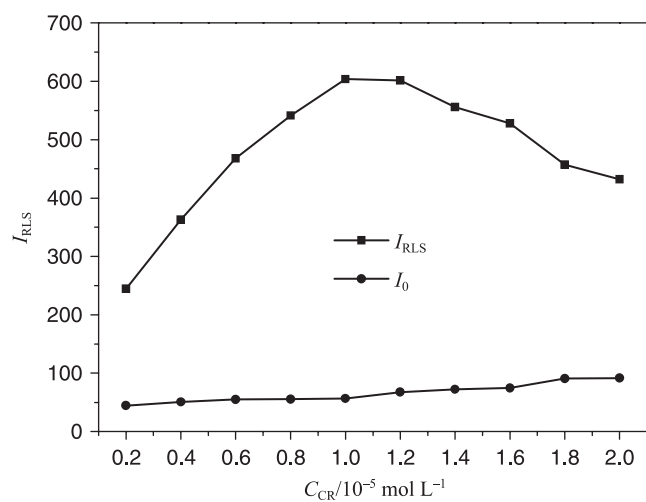


Figure 4. Effect of CR concentration on the RLS intensity. Conditions— C_{QDS} $6.0 \mu\text{g mL}^{-1}$, pH 4.5.

maximum. In addition, we tested a suitable amount of a BR buffer solution for the system. The results showed that 0.8–1.2 mL of BR buffer solutions were optimum. Therefore, a BR buffer solution with pH 4.5 was used in this research and the optimum volume of buffer was 1.0 mL.

Effect of the CR concentration

As shown in Fig. 4, the effect of CR concentration on the RLS intensity of this system was studied. If the concentration of CR was too low, the enhanced RLS intensity was small because of the incomplete reaction between QDS and CR. On the contrary, if the concentration of CR was too high, the scattering intensity was also faint due to the self-assembling of CR. Therefore, $1.0 \times 10^{-5} \text{ mol L}^{-1}$ CR was used in this research.

Effect of salt effect

The effect of salt on the RLS intensity of the system is presented clearly in Fig. 5. It can be observed from Fig. 5 that the RLS

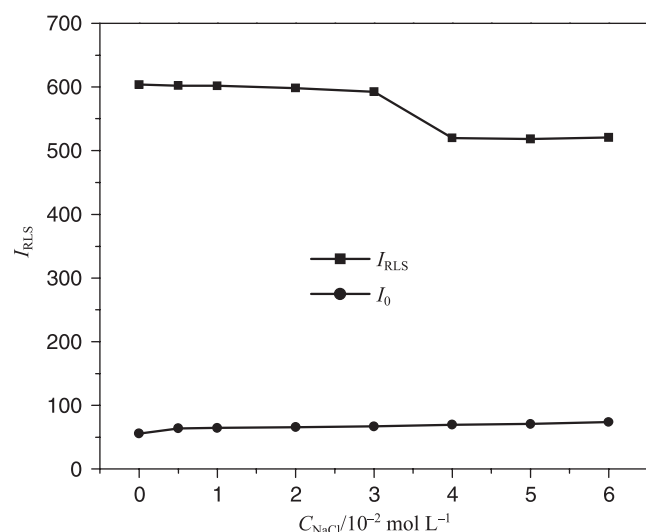


Figure 5. Effect of ionic strength on the RLS intensity. Conditions— C_{QDS} $6.0 \mu\text{g mL}^{-1}$, C_{CR} $1.0 \times 10^{-5} \text{ mol L}^{-1}$, pH 4.5.

intensity of the system QDS-CR decreases gradually with the increasing NaCl concentration. When NaCl concentration reaches $3.0 \times 10^{-2} \text{ mol L}^{-1}$, the RLS signal maintains a constant value. The variation of RLS signal with ionic strength indicates that electrostatic interaction plays a dominant role in this reaction. When the concentration of the NaCl increases, the effects of the electrostatic shielding of charges between CR and QDS reduces the combination between QDS and CR and results in a decreased RLS signal. Therefore, this system should be under a low ionic strength condition without adding NaCl.

Effect of addition order

The effect of the addition order of the reagents on the intensity was investigated. The result shows that mixing buffer solution and CR solution first and then adding QDS can give a higher intensity than other sequences of adding the reagents. This may be explained by the buffer and CR solution mixing first and promoting the ionization of CR, benefiting the reaction of QDS with CR. Therefore, in this research, the optimal order for addition of the reagents was: buffer-CR-QDS.

Incubation time and stability

The stability of the QDS-CR system was investigated by measuring the RLS intensity every 2 min for 2.5 h immediately after mixing. The results show that the formation time of all reaction products is 20 min at room temperature and the RLS intensity will remain constant over 120 min (change of RLS intensity within $\pm 5\%$).

Selectivity of the method

Under the optimum conditions, the effects of some foreign coexisting substances on the determination of $6.0 \mu\text{g mL}^{-1}$ were investigated by pre-mixing QDS with foreign substances and the results are listed in Table 1. It can be seen from Table 1 that sugars, urea, amino acids and some ions in fluids can be allowed with high concentration. Therefore, the selectivity of the method is good and the method can be applied to determine QDS in practical samples.

Calibration and detection limit

According to the above standard procedure, the RLS intensities were obtained under optimum conditions and a calibration curve was constructed. The linear range was $0.2\text{--}8.4 \mu\text{g mL}^{-1}$, and the linear regression equation was calculated as $\Delta I_{RLS} = -84.9 + 105.1C$ ($\mu\text{g mL}^{-1}$) with regression coefficient $r = 0.9987$ ($n = 6$). The detection limit was 12.0 ng mL^{-1} . The limit of detection is given by $3S_0/S$, where 3 is the factor at the 99% confidence level, S_0 is the standard deviation of the blank measurements ($n = 11$) and S is the slope of the calibration curve. Comparisons with some other assays of QDS are shown in Table 2. The results show that the proposed assay method exhibits simplicity, high sensitivity and a wide linear range. This method provides a sufficient sensitivity for the one-step measurement of trace amounts of QDS in solution samples.

Mechanism of the reaction

CR, a kind of bisazo anionic dye, is negatively charged in aqueous solution due to two sulfonic acid groups within its structure,

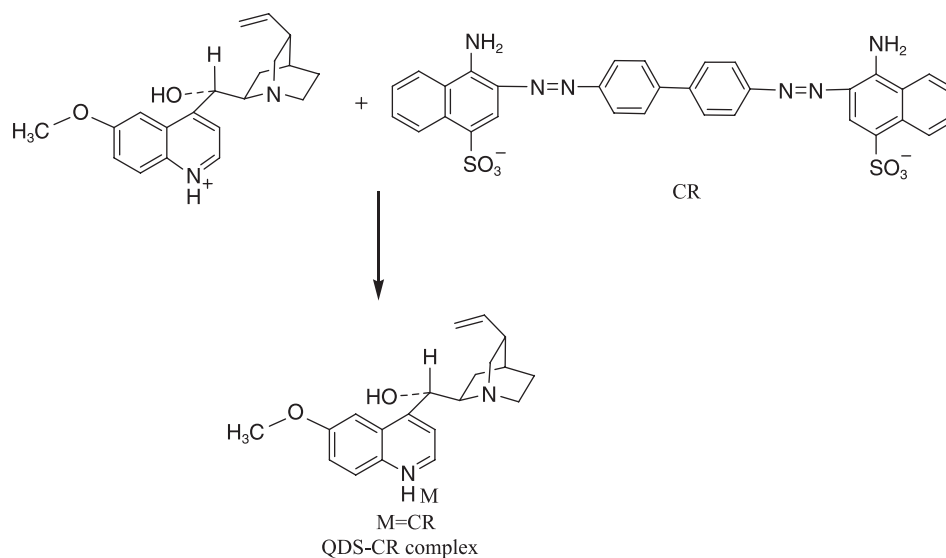
Table 1. Effects of foreign substances (conditions: pH 4.5, $C_{CR} 1.0 \times 10^{-5} \text{ mol L}^{-1}$, $C_{QDS} 6.0 \mu\text{g mL}^{-1}$)

Foreign substances	Concentration of foreign substances ($\mu\text{g mL}^{-1}$)	Relative error (%)	Foreign substances	Concentration of foreign substances ($\mu\text{g mL}^{-1}$)	Relative error (%)
Na(I)	900	-2.8	Serine	500	4.8
K(I)	1800	-3.9	Glycin	90	4.6
Mg(II)	60	1.7	Phenylalanine	600	4.1
Ca(II)	900	3.8	Lysine	72	3.6
Cd(II)	150	2.4	Arginine	100	-3.8
Zn(II)	120	3.4	Alanine	600	-4.0
Fe(II)	3	3.0	Aspartic acid	300	-0.3
NO_3^-	230	3.4	Valine	480	3.0
HCO_3^-	300	-4.3	Saccharose	1500	4.0
Cl^-	1200	-2.8	Glucose	3000	4.7
SO_4^{2-}	240	1.7	Fructose	1500	3.8
Methionine	500	3.3	Lactose	1500	0.5
Cystenine	100	-0.7	Leucine	480	-2.3
Tryptophan	300	-0.9	Starch	30	0.9
Tyrosine	100	-3.6	Vitamin C	150	0.8
Histidine	100	-3.8	Urea	120	3.7

Table 2. Comparisons with some other assays of QDS

Method	Experimental conditions	Determination limit (ng mL^{-1})	References
FLU ^a	Sulfuric acid, $\lambda_{\text{ex}}/\lambda_{\text{em}} = 353 \text{ nm}/445 \text{ nm}$, extraction by benzene	10.0	[5]
HPLC	Fluorescent detection	2.4	[6]
CE	Laser-induced fluorescence detection	24.1	[7]
FMA	Poly(vinyl alcohol)-124	20.4	[8]
RLS	Congo red, 562 nm	12.0	This work

^aThe linear range of FLU is 0.06–0.8 $\mu\text{g mL}^{-1}$.

**Figure 6.** The formed process of the supermolecular complex between QDS and CR.

shown in Fig. 6. Therefore, it will ionize as an anion in aqueous solution. On the other hand, QDS will ionize as a cation in aqueous solution. Before interaction, either CR anion or QDS cation has strong hydrophilicity. When the hydrophobicity is enhanced

owing to the neutralization of charges and the appearance of the liquid–solid interface, they react with each other to form an ion-association complex, which can be further demonstrated by absorption and fluorescence spectra. The formation of the

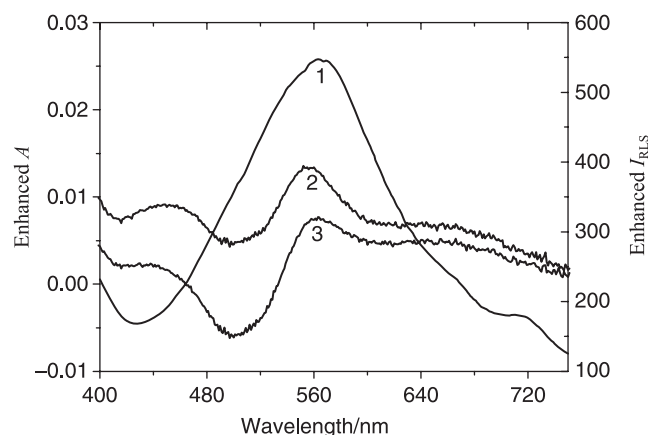


Figure 7. Comparisons between RLS and absorption spectra. Conditions—(1) RLS spectra (enhanced I_{RLS}): C_{CR} 2.0×10^{-6} mol L $^{-1}$; C_{QDS} $6.0 \mu\text{g mL}^{-1}$; pH 4.5; (2) absorption spectra against the reagent blank (enhanced A): C_{CR} 2.0×10^{-6} mol L $^{-1}$; C_{QDS} $8.0 \mu\text{g mL}^{-1}$; pH 4.5; (3) absorption spectra against the reagent blank: C_{CR} 2.0×10^{-6} mol L $^{-1}$; C_{QDS} $6.0 \mu\text{g mL}^{-1}$; pH 4.5.

hydrophobic interface enhances the RLS intensity. The formation process of the supermolecular complex between QDS and CR is shown in Fig. 6. From comparisons between RLS and absorption spectra, shown in Fig. 7, it can be seen that the RLS spectra of complex for QDS–CR is situated near their absorption band. RLS is an absorption rescattering process, which is produced when resonance takes place between the RLS and the light absorption with equal frequency.^[29,32] Therefore, the RLS peak (562 nm) is close to the corresponding absorption peak (560 nm). In this case, a resonance scattering effect is produced, which leads to the enhanced RLS intensity.

The fluorescence excitation and emission spectra of QDS reacting with variable CR concentrations are shown in Fig. 8. It can be seen from Fig. 8 that two excitation peaks are located at 254 and 350 nm and one emission peak is located at 451 nm. Figure 8 shows the fluorescence emission spectra of QDS reacting with variable CR concentrations which were operated by 254 nm of the maximum excitation wavelength. With the various amounts of CR added, a decrease in fluorescence intensity could be observed, which might be attributed to the supermolecular complex between QDS and CR. This phenomenon indicates that interaction between QDS and CR occurs, which is beneficial to the RLS intensity enhancement. Therefore, from the absorption, fluorescence and RLS spectra shown above, it can be con-

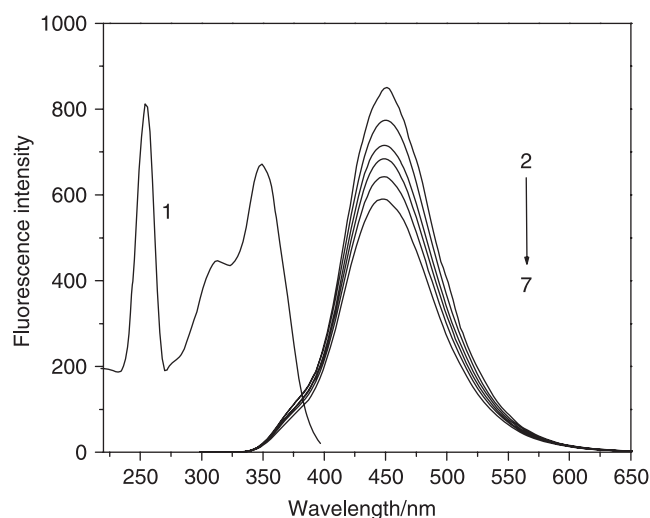


Figure 8. The fluorescence excitation and emission spectra of QDS reacting with variable CR concentrations. Conditions— C_{QDS} $6.0 \mu\text{g mL}^{-1}$, C_{CR} ($\times 10^{-5}$ mol L $^{-1}$): (1–2) 0; (3) 0.2; (4) 0.4; (5) 0.6; (6) 0.8; (7) 1.0, pH 4.5.

cluded that the ion-association complex between QDS and CR is formed by function of electrostatic and hydrophobic interaction force, which greatly enhances the RLS intensity.

Analytical application

In order to evaluate the applicability and reliability of the proposed method, the present method was applied to determine QDS in human serum and urine samples using the standard addition method. In addition, the proposed method was also used for the determination of QDS in simulated serum samples. The concentration of each component of simulated serum samples was chosen to match its normal level in human serum.^[33] The results are listed in Table 3. The relative standard deviation (RSD) and recovery were examined by using the standard addition method. It can be found from Table 3 that the RLS method has a good repeatability. The RSD of serum is 4.2–2.8%. That of the human urine is 3.2–2.3%. The recovery of serum is 102.2–100.6%. That of human urine is 99.0–100.4%. The results showed that this simple, fast and sensitive method can be successfully applied to the determination of QDS in urine and simulated human serum samples.

Table 3. The results for determination of QDS in serum and urine samples (conditions: pH 4.5, C_{CR} 1.0×10^{-5} mol L $^{-1}$)

Sample	Found ($\mu\text{g mL}^{-1}$)	Added ($\mu\text{g mL}^{-1}$)	Total found mean ($n = 5$) ($\mu\text{g mL}^{-1}$)	Recovery (%)	RSD ($n = 5$) (%)
Serum 1	ND ^a	2.0	2.02	101.0	3.7
Serum 2	ND	5.0	5.03	100.6	2.8
Urine 1	ND	2.0	1.98	99.0	3.2
Urine 2	ND	5.0	5.02	100.4	2.3
SMSS ^b 1	2.2	2.0	4.26	101.4	4.2
SMSS 2	5.3	1.0	6.44	102.2	3.6

^aNot detected; ^bsimulated serum sample.

Conclusions

In weak acidic conditions, QDS reacts with CR to form an ion-association complex, which results in the enhanced RLS intensity. Based on this, a sensitive method was developed for the determination of QDS by RLS technique in this paper. The effects of some interacting parameters on the enhanced RLS intensity were studied and optimized. The proposed method enables simple, sensitive and rapid determination of QDS and has been successfully applied to the determination of QDS in urine and simulated human serum samples with satisfactory results.

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