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Resonance Rayleigh Scattering Spectra, Non-Linear Scattering Spectra of Congo Red-Copper(II)-Protein System and Its Analytical Application

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A method to determine protein concentrations by using Congo red (CR)-Cu(II) complex as a spectral probe has been developed. In pH 6 Britton-Robinson buffer medium, the CR-Cu(II) complex could react with proteins such as bovine serum albumin, human serum albumin and ovalbumin to form the ternary ion-association complexes by virtue of electrostatic attraction and the hydrophobic force, which resulted in a great enhancement of resonance rayleigh scattering and resonance non-linear scattering such as second-order scattering and frequency doubling scattering. The maximum scattering peak was located at 278 nm for resonance rayleigh scattering, 542 nm for second-order scattering and 390 nm for frequency doubling scattering, respectively. The enhancements of the three scattering intensities (ΔI) were directly proportional to the concentration of proteins. The detection limits (3δ) for the three proteins were 3.57-5.74 ng mL^{-1} for resonance rayleigh scattering method, 9.46-12.25 ng mL^{-1} for second-order scattering, 14.35-19.26 ng ml^{-1} for frequency doubling scattering method, respectively. Among them the resonance rayleigh scattering method exhibited the highest sensitivity and the bovine serum albumin system was more sensitive than other protein systems. The optimum condition such as the pH, the ion strength and the concentration of the probes for the reaction was investigated. In addition, the enhancement reasons of resonance rayleigh scattering, frequency doubling scattering and second-order scattering of the system are also studied. The present method has been applied to the determination of total proteins in human urine and serum samples with satisfactory results.

Keywords: Congo red, Protein; Cu(II), Resonance rayleigh scattering, Non-linear scattering spectra.

INTRODUCTION

Proteins, the essential "factories" within living systems, are the essential component of organism, playing an important role in several vital activities, such as enzyme catalysis, immune protection, signal transduction, membrane transport and electron transfer, *etc*. Hence, the quantitative determination of protein is considerably essential in many areas, such as biochemistry, pharmaceutical studied and clinical medicine[1].

A number of traditional methods have been proposed over the years for the determination of proteins, such as the Bradford method[2], Lowry method[3], Coomassie brilliant blue (CBB) method[4], bromophenol blue method[5], bromocresol green method[6], *etc*. Unfortunately, there is no universal methodology for all media. Because of the complicated composition and the low contents of proteins in biological samples, the methods above also have some shortcomings, *e.g*., the Lowry method allows determination of proteins at concentration higher than 10 μg mL^{-1}. The bromophenol blue and bromocresol green methods are tedious owing to their cumbersome operating steps, poorly sensitive and are seriously interfered by various coexisting substances such as thiol reagents, some metal ions and amino acids[7]. The coomassie brilliant blue assay, although sensitive, is inconvenient in application due to the non-linear calibration graph and limited stability of the coomassie brilliant blue-protein complex[2,4]. Therefore, it is still a worthwhile subject to further study and develop more sensitive, simple and selective methods for the determination of trace amount of protein.

To overcome these limitations of the above, the new analytical technique of resonance rayleigh scattering (RRS) and resonance nonlinear scattering (RNLS) such as second order scattering (SOS) and frequency doubling scattering (FDS) have been widely used for the investigation and determination of proteins, because of their high sensitivity, simplicity, quickness and its potential for providing real-time measurements. They mainly based on ion association reaction between small molecules such as dye probe[8], metal probe[9,10], metal complex probe[11,12] and proteins, association particles reaction between the anionic ligand[13], organic probe[14-16] and proteins. Since

organic dyes can serve as effective probes of the structures and functions of biological macromolecules, interest has been raised in the study of the interaction of dyes with proteins in recent decades. Various new dye binding methods have been put forward for protein analysis by spectrophotometric and fluorometric methods[17-19].

Congo red (diphenyl-4,4'-*bis*(azo-2-)-1-naphthylamine-4-sulfonate sodium), is a common pH indicator. Because of their excellent qualities, the mechanism of the combination of azo dye probes and protein have been focused by more and more workers[20-22]. However, the study which uses resonance rayleigh scattering and resonance non linear scattering technology with the help of dye-metal complex probe to determine trace protein has not been reported. In this paper, we first studied the characteristics of resonance rayleigh scattering, second-order scattering and frequency doubling scattering spectra of CR-Cu(II)-protein system. In the Britton-Robinson buffer solution, protein negative charge-contained molecules were bounded to the surface of CR-Cu(II) composite which contained positive charge *via* electrostatic attraction and hydrophobic interaction forces and formed an ion-association complex. It can enhance the resonance rayleigh scattering, second-order scattering and frequency doubling scattering intensity of the reaction system, the enhancements of the three scattering intensity (ΔI) were directly proportional to the concentration of proteins over a wide range and their detection limits are at nanogram level. Based on this a new assay for the determination of protein is described and the detection limits for bovine serum albumin, human serum albumin and ovalbumin are all down to 20 ng mL^{-1}. Little interference from most co-existing substances was observed. The reasons of enhancement for resonance rayleigh scattering, second-order scattering and frequency doubling scattering spectra were also discussed. The method was applied to the determination of proteins in human urine and serum samples with satisfactory results.

EXPERIMENTAL

An F-7000 fluorescence spectrophotometer (Tokyo, Japan) was used to record the resonance rayleigh scattering, second-order scattering and frequency doubling scattering spectra and measure the scattering intensity with the slits (EX/EM) of 5.0/5.0 nm for resonance rayleigh scattering and the slits (EX/EM) of 5.0/5.0 nm for second-order scattering and frequency doubling scattering. PMT voltage was 400 V. A UV-8500 spectrophotometer (Tianmei, Shanghaii, China) was used to record the absorption spectra and measure absorbance intensity. A PHS-3C meter (Leici, Shanghai, China) was used to adjust the pH values.

Bovine serum albumin (BSA, Sino-American Biotechnology Company), human serum albumin (HAS, Sino-American Biotechnology Company) and ovalbumin (Ova, Sino-American Biotechnology Company) were directly dissolved in water to prepare stock solutions of 250 mg L^{-1} each and stored at 0-5 °C. The working solution was further diluted with water to 1.0 mg L^{-1} prior to using. In this study, Congo red, was used as probe for protein determination. Congo red was purchased from shanghai Chemical Reagent Co., China. It was dissolved in water to prepare a stock solution of 1.0×10^{-3} mol L^{-1}. A Cu(II) standard solution of 1×10^{-4} mol L^{-1} was prepared by dissolving $Cu(NO_3)_2$ in water. Britton-Robinson buffer solutions with different pH were prepared according to suitable proportion and different pH values with a pH meter. Unless mentioned otherwise, all reagents used were analytical reagent grade (A.R.) and doubly distilled water was used throughout.

Experimental procedure: A certain amount of proteins, 1.25 mL of Britton-Robinson buffer solution with pH 6, 1 mL of 1×10^{-3} mol L^{-1} congo red solution and 1 mL of 1×10^{-4} mol L^{-1} Cu(II) solution were added into a 10 mL colorimetric tube respectively. The mixture was then diluted to the mark with water and mixed thoroughly. After incubating for 10 min, the resonance rayleigh scattering spectra of the system were recorded with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ ($\Delta\lambda = 0$ nm) using the Hitachi F-7000 spectrofluorophotometer. The frequency doubling scattering intensity (I_{FDS}) and the second-order scattering intensity (I_{SOS}) of the system produced at different incident wavelength were recorded at $\lambda_{em} = 1/2\lambda_{ex}$ and $\lambda_{em} = 2\lambda_{ex}$, respectively. The frequency doubling scattering and second-order scattering spectrum were obtained by plotting the different wavelength against the frequency doubling scattering intensity (I_{FDS}) and the second-order scattering intensity (I_{SOS}). Then, the scattering intensity of I_{RRS}, I_{SOS} and I_{FDS} for the reaction product and I_{0RRS}, I_{0SOS} and I_{0FDS} for the reagent blank at their own maximum wavelengths, $\Delta I = I - I_0$, were measured and the absorption spectra was recorded simultaneously.

RESULTS AND DISCUSSION

Fig. 1 shows the resonance rayleigh scattering spectrum of CR-Cu(II)-BSA system which was recorded by synchronous scanning at $\lambda_{ex} = \lambda_{em}$ from 220 to 600 nm. From Fig. 1a, it was clear that the resonance rayleigh scattering intensities of congo red, bovine serum albumin, Cu(II), CR-Cu(II), CR-BSA and BSA-Cu(II) were very weak under the same measurement conditions. The weak resonance rayleigh scattering of CR-Cu(II) could be enhanced greatly by addition of micro amounts of proteins, resulting in two characteristic peaks in the wavelength range 220-600 nm. From Fig. 1b, it was clear that the enhancement of resonance rayleigh scattering intensity (Δ_{IRRS}) for CR-Cu(II)-BSA system was directly proportional to the concentrations of bovine serum albumin at the maximal wavelength of 278 nm. Hence, the resonance rayleigh scattering method could be applied to determination of bovine serum albumin.

Second-order scattering and frequency doubling scattering spectra: Figs. 2 and 3 show the second-order scattering and frequency doubling scattering spectra of the congo red, Cu(II), bovine serum albumin and their binding product, respectively. Here, λ_1 was the incident wavelength, λ_{SOS} was the second-order scattering wavelength and λ_{FDS} was the frequency doubling scattering wavelength. It could be seen that, under the experimental conditions, intensities of second-order scattering and frequency doubling scattering of congo red, bovine serum albumin, Cu(II), CR-Cu(II), CR-BSA and BSA-Cu(II) were very weak and could not be used to analyze. When congo red, Cu(II) mixed with bovine serum albumin to

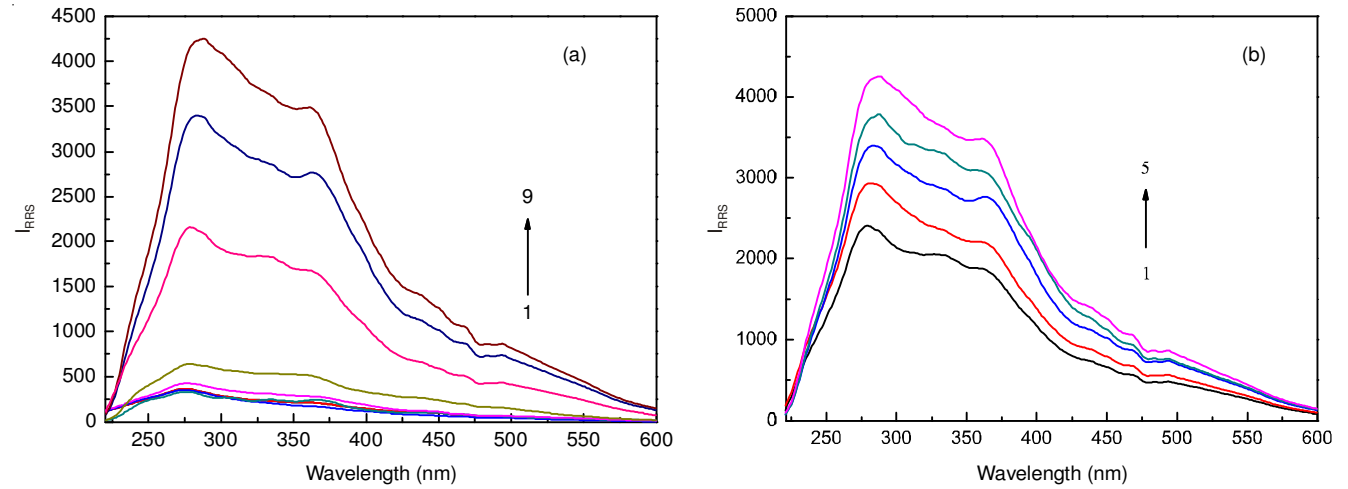

Fig. 1. Resonance rayleigh scattering spectra; (a) Resonance rayleigh scattering spectra of the CR-Cu(II)-protein system; (1) Congo red; (2) BSA; (3) Cu(II); (4) CR-BSA; (5) CR-Cu(II); (6) BSA-Cu(II); (7) CR-Cu-HSA; (8) CR-Cu-OVA; (9) CR-Cu-BSA; (b) The relationship between resonance rayleigh scattering intensity and the concentration of bovine serum albumin. [Bovine serum albumin]: 1, 2, 3, 4, 5 μg mL^{-1}. Experimental conditions: [congo red]: 1×10^{-4} mol L^{-1}; [Cu(II)]: 1×10^{-5} mol L^{-1}; pH 6

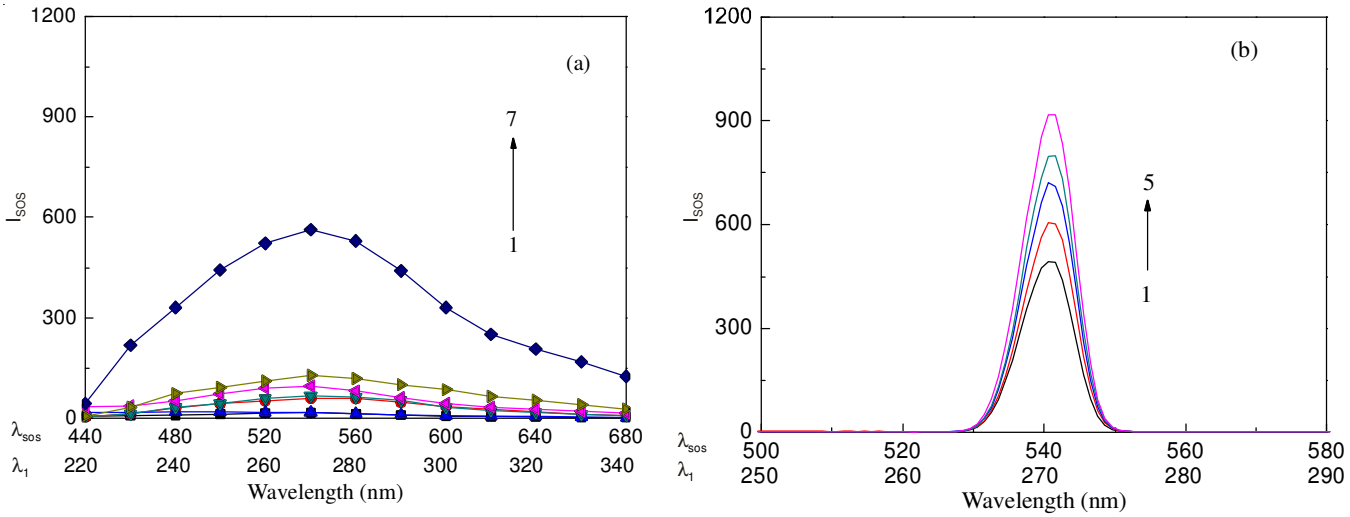

Fig. 2. Second-order scattering spectra; (a) second-order scattering spectra of CR-Cu(II)-BSA system; (1) congo red; (2) BSA; (3) Cu(II); (4) CR-Cu(II); (5) CR-BSA; (6) BSA-Cu(II); (7) CR-Cu(II)-BSA; (b) The relationship between second-order scattering intensity and the concentration of bovine serum albumin. [Bovine serum albumin]: 1, 2, 3, 4, 5 μg mL^{-1}. Experimental conditions: [congo red]: 1×10^{-4} mol L^{-1}; [Cu(II)]: 1×10^{-5} mol L^{-1}; pH 6

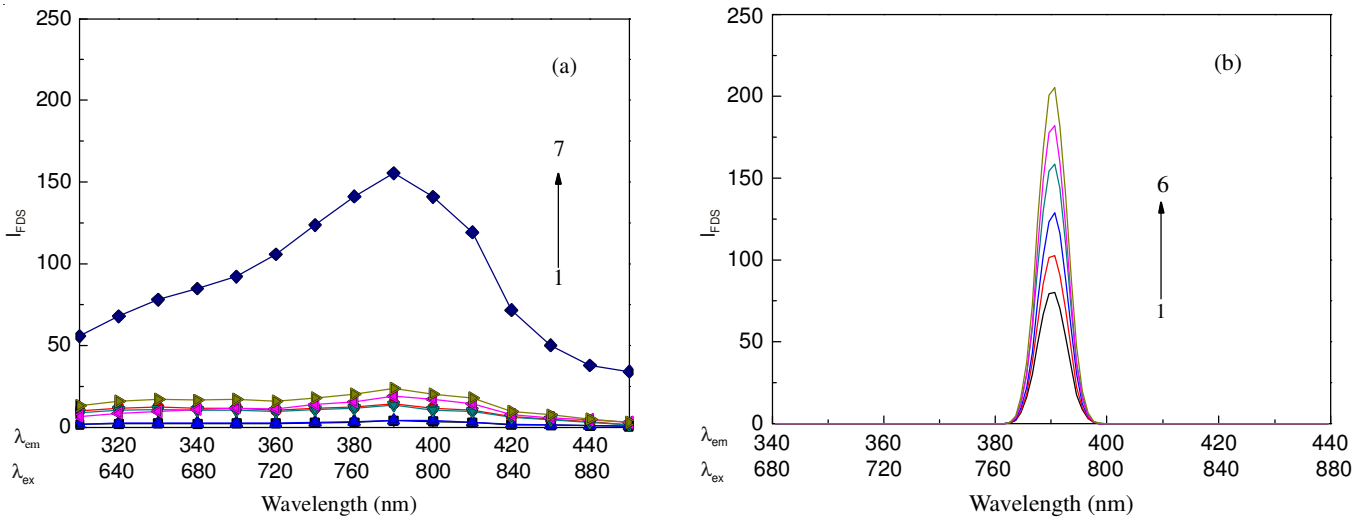

Fig. 3. Frequency doubling scattering spectra; (a) frequency doubling scattering spectra of CR-Cu(II) -BSA system; (1) congo red; (2) BSA; (3) Cu(II); (4) CR-Cu(II); (5) CR-BSA; (6) BSA-Cu(II); (7) CR-Cu(II)-BSA; (b) The relationship between frequency doubling scattering intensity and the concentration of bovine serum albumin. [Bovine serum albumin]: 1, 2, 3, 4, 5, 6 μg mL^{-1}. Experimental conditions: [congo red]: 1×10^{-4} mol L^{-1}; [Cu(II)]: 1×10^{-5} mol L^{-1}; pH 6

form ion-complexes, the intensities of second-order scattering and frequency doubling scattering were enhanced remarkably. The characteristics of the two spectra were similar. As shown in Figs. 2a and 3a, the maximum scattering peaks were located at 542 nm for second-order scattering and 390 nm for frequency doubling scattering, respectively. Shown in Figs. 2b and 3b, the enhancement of second-order scattering and frequency doubling scattering intensities of the CR-Cu(II)-BSA system were directly proportional to the concentration of bovine serum albumin in a certain range. Therefore, the second-order scattering and frequency doubling scattering method could be applied to the determination of the investigated protein.

Optimum reactive conditions: Resonance rayleigh scattering, second-order scattering and frequency doubling scattering methods all were suitable for the trace determination of bovine serum albumin. But the sensitivity of resonance rayleigh scattering method was much higher than those of second-order scattering and frequency doubling scattering methods. Therefore, the following experiments such as optimum reaction conditions, the influence of coexisting substances and the analytical application were mainly investigated by taking resonance rayleigh scattering for example.

Effect of the solution acidity: The influence of different kinds of buffer medium were tested, such as Britton-Robinson buffer solution, $CH_3COOH + CH_3COONa$ buffer solution, Na_2HPO_4-KH_2PO_4 buffer solution on the resonance rayleigh scattering intensity of the reaction system. The results show that the sensitivity and stability of the Britton-Robinson buffer were the best among the buffers. Therefore, the Britton-Robinson buffer solution was selected to control the pH of solution.

As shown in Fig. 4, the resonance rayleigh scattering intensity enhancement of bovine serum albumin was seriously affected by acidity and it reached a maximum at 6, therefore this pH was chosen for the assay. The resonance rayleigh scattering intensities would decrease if the pH values were beyond these ranges. The appropriate amount of buffer solution was 1.25 mL.

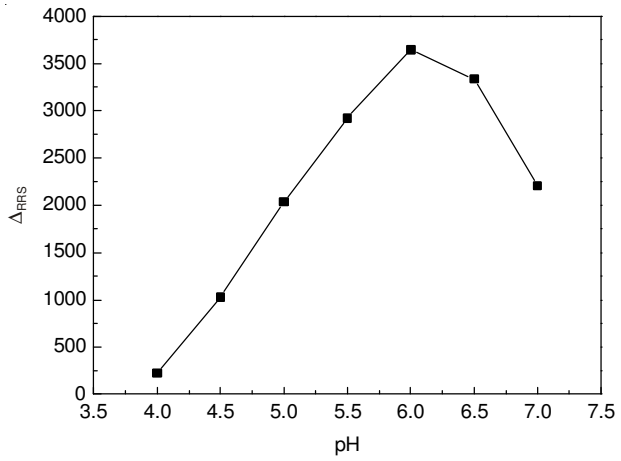

Fig. 4. Effect of solution acidity on I_{RRS} of CR-Cu(II)-BSA system. (pH: 4, 4.5; 5; 5.5; 6; 6.5; 7). Experimental conditions: C(BSA): 2 μg mL^{-1}; C(congo red): 1×10^{-4} mol L^{-1}; C[Cu(II)]: 1×10^{-5} mol l^{-1}.

Effect of the congo red concentration: The effect of congo red concentration on the resonance rayleigh scattering intensities of the system was investigated. The results shown in Fig. 5 revealed that under the optimum experimental conditions, the formation of the ion-association complex molecular particle increased with the addition of congo red, which enhanced Δ_{IRRS} gradually. When the concentration of congo red was 1×10^{-4} mol L^{-1}, Δ_{IRRS} reached the maximum value. If the amount of congo red was not enough, the reaction would not be finished. If the amount of congo red was excessive, congo red aggregated by it, Δ_{IRRS} would decrease. As a result, 1×10^{-4} mol L^{-1} of congo red was chosen for the optimum concentration.

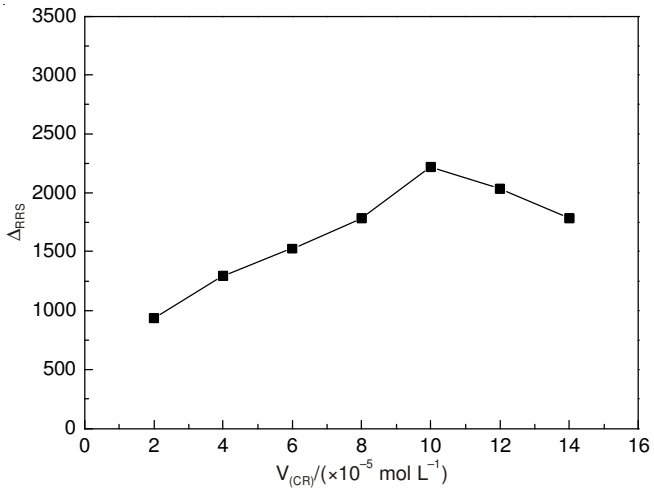

Fig. 5. Effect of congo red addition on I_{RRS} of the CR-Cu(II)-BSA system; Experimental conditions: C(BSA): 2 μg mL^{-1}; C(congo red): 2×10^{-5}, 4×10^{-5}, 6×10^{-5}, 8×10^{-5}, 1×10^{-4}, 1.2×10^{-4}, 1.4×10^{-4} mol L^{-1}; C[Cu(II)]: 1×10^{-5} mol L^{-1}; pH: 6

Effect of Cu(II) concentration: The experimental results showed that resonance rayleigh scattering intensity reached the maximum and retained stability when the concentration of Cu(II) was from 8×10^{-6} to 1.2×10^{-5} mol L^{-1} (Fig. 6). So the optimum experiment concentration of Cu(II) was 1×10^{-5} mol L^{-1}.

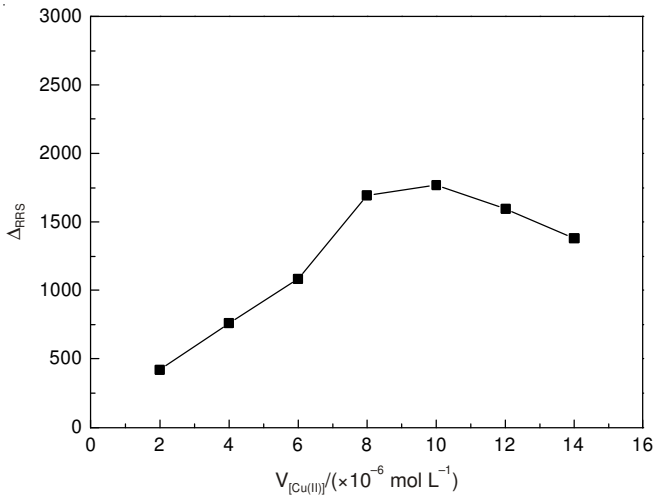

Fig. 6. Effect of Cu(II) addition on I_{RRS} of CR-Cu(II)-BSA system. Experimental conditions: C(BSA): 2 μg mL^{-1}; C(congo red): 1×10^{-4} mol L^{-1}; C[Cu(II)]: 2×10^{-6}, 4×10^{-6}, 6×10^{-6}, 8×10^{-6}, 1×10^{-5}, 1.2 × 10-5, 1.4×10^{-5} mol L^{-1}; pH: 6

Reaction speed and the stability: Under the room temperature, the reaction could complete in 5 min and the resonance rayleigh scattering intensity would be kept constant in 10 h at least. Therefore, this experiment was carried out after 10 min.

Effect of ionic strength: The effect of ionic strength on the intensity of resonance rayleigh scattering for the CR-Cu(II)-BSA system was investigated by using different concentration of NaCl solutions. The results show that it had little effect on the resonance rayleigh scattering when NaCl concentration was lower than 1×10^{-2} mol L^{-1}, but resonance rayleigh scattering intensity would gradually decreased with further increasing in NaCl concentration. It revealed that with the increase of NaCl concentration, the presence of large amounts of Cl^- and Na^+ destroyed the complex of the charge balance, causing the reaction sensitivity decreasing[23].

Enhancement reasons of resonance rayleigh scattering, frequency doubling scattering and second-order scattering

Resonance enhanced rayleigh scattering effect: Resonance rayleigh scattering is an absorption-rescattering process produced by the resonance between Rayleigh scattering and light absorption with equal frequency when the wavelength of Rayleigh scattering is located at its absorption spectrum. Therefore, resonance rayleigh scattering spectrum is closely related to the absorption spectrum. Fig. 7 shows the comparison of resonance rayleigh scattering and absorption spectra of CR-Cu(II)-BSA system. It could be seen that resonance rayleigh scattering peak is located at its absorption band. The resonance rayleigh scattering peaks have good corresponding relationship with the absorption peaks, which results in a resonance enhanced effect and leads to great enhancement of resonance rayleigh scattering intensity.

Increase of the molecular volume: The molecular weight of congo red is 696.66 before the reaction, however, when CR-Cu(II) reacted with bovine serum albumin to form the ion-association complex, the total molecular weight increased largely. The increase in the molecular volume (or weight) is a significant factor to the enhancement of the resonance rayleigh scattering intensity.

Hydrophobic effect: Before reaction, congo red, Cu(II) or bovine serum albumin alone is soluble and stable in solution. The scattering of the solutions is very low. congo red and Cu(II) can become a cationic complex of CR-Cu(II) under the existence of buffer without protein. When the cationic complex is close to protein, the CR-Cu(II) will combine with protein to become the protein-CR-Cu(II) complex due to the electrostatic attractions of the negative charge of protein and the positive charge of CR-Cu(II). In this case, the CR-Cu(II) molecule binds on the surface of protein by virtue of a positive-negative electric field and their charges are neutralized and the hydrophobicity of the molecule was enhanced greatly. The aggregation of the chromophores of the cationic CR-Cu(II) complex on the biological macromolecule will greatly enhance the intensity of resonance rayleigh scattering after the cationic complex combines with protein[24].

Affected by all the above factors, the intensities of resonance rayleigh scattering and non-linear scattering will enhanced notably.

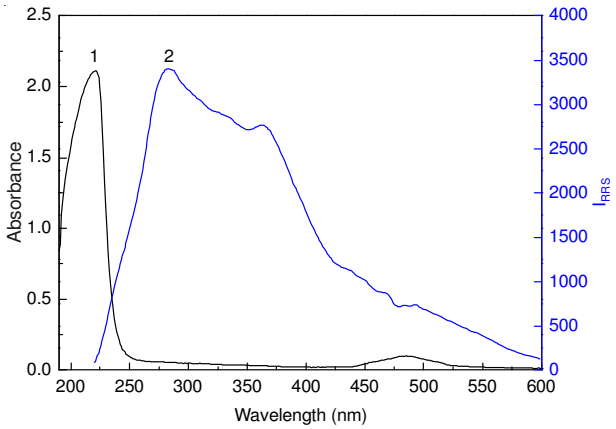

Fig. 7. Comparison of resonance rayleigh scattering spectrum with absorption spectrum for the system; 1. Absorption spectrum (against reagent blank); 2. resonance rayleigh scattering spectrum. Experimental conditions: C(BSA): 2 μg mL^{-1}; [congo red]: 1×10^{-4} mol L^{-1}; [Cu(II)]: 1×10^{-5} mol L^{-1}; pH 6

Application of method

Calibration curves and sensitivity: Under the optimum experimental conditions, the relative scattering intensities Δ_{IRRS}, Δ_{ISOS} and Δ_{IFDS} of the ion-association complex were measured at their maximum scattering wavelengths. The calibration graphs of Δ_{IRRS}, Δ_{ISOS} and Δ_{IFDS} against concentration of protein were plotted. The correlation parameter was listed in Table-1. It was evident that the methods for the determination of protein had a high sensitivity, ΔI was directly proportional to the concentration of protein in a certain range and the determination limits (3σ) of these methods were in the range of 3.57-19.26 ng mL^{-1}. Comparing the detection limits of the three methods, it was obvious that the resonance rayleigh scattering method had the highest sensitivity. Therefore, the resonance rayleigh scattering

TABLE-1
STANDARD REGRESSION EQUATION OF PROTEINS

Method	Proteins	Regression equation (c, μg mL^{-1})	Correlation coefficient	Linear range (c, μg mL^{-1})	Determination limit (c, ng mL^{-1})
Resonance rayleigh scattering	Bovine serum albumin	ΔI = 495.56c + 699.89	0.9988	0.015-6.5	3.57
	Human serum albumin	ΔI = 488.78c + 649.46	0.9986	0.015-6.5	4.16
	Ovalbumin	ΔI = 466.45c + 614.92	0.9985	0.025-5.0	5.74
Second-order scattering	Bovine serum albumin	ΔI = 127.16c + 438.82	0.9986	0.015-6.0	9.46
	Human serum albumin	ΔI = 117.06c + 447.82	0.9992	0.020-6.0	11.58
	Ovalbumin	ΔI = 96.06c + 378.89	0.9991	0.045-6.5	12.25
Frequency doubling scattering	Bovine serum albumin	ΔI = 28.65c + 40.78	0.9992	0.025-6.0	14.35
	Human serum albumin	ΔI = 27.08c + 23.23	0.9991	0.025-6.0	19.21
	Ovalbumin	ΔI = 17.67c + 25.84	0.9989	0.030-5.5	19.26

method was taken as an example for studying the effects of coexistent substances. According to the experimental method, we detected bovine serum albumin solution (2 μg mL^{-1}) for 11 times and its relative standard deviation (RSD) was 0.8 %.

Selectivity of the method: Under the optimum conditions, taking the resonance rayleigh scattering method as an example, when the relative error was lower than ± 5 % and bovine serum albumin was about 2 μg mL^{-1}, the effect of some potential coexisting substances on the determination of bovine serum albumin was investigated and the results were given in Table-2. We show that in the presence of protein (2 μg mL^{-1}), high concentrations of various metal ions and acid radical anions do not interfere with the assay. These results indicated that this method has good selectivity and could be applied to the determination of protein in some samples.

Sample determination

Determination of proteins in synthesized samples: The concentrations of bovine serum albumin in four synthesized samples were determined using the resonance rayleigh scattering method and the results were listed in Table.-3. It could be seen that the recovery of protein was 97-101 % and the relative standard deviation were in all instances less than 3.1 %.

Determination of proteins in human serum and urine samples: The present method was applied to determine total protein in human serum and urine. Table-4 shows the results which were very close to those obtained by the Bradford method. Therefore the determination of protein by this method was reliable, sensitive and practical.

TABLE-4
ANALYTICAL RESULTS FOR HUMAN SERUM AND URINE SAMPLES (n = 6)

Sample	This method		Coomassie brilliant blue assay	
	Found[a]	RSD (%)[b]	Found[a]	RSD (%)[b]
Serum 1	76.6	2.6	77.9	2.2
Serum 2	83.1	2.1	84.7	2.4
Serum 3	86.5	2.5	87.5	1.9
Urine 1	49.6	2.7	51.4	2.1
Urine 2	54.4	1.7	55.2	2.5
Urine 3	56.8	2.3	57.6	2.4

[a]Mean protein concentration in serum is expressed in mg mL^{-1} and that in urine is in mg L^{-1}, [b]Relative standard deviation

Conclusion

In the weak acidic medium of Britton-Robinson buffer solution (pH= 6), congo red, Cu(II) interacted with proteins to form a ternary ion-association complex, which enhanced the intensities of resonance rayleigh scattering, second-order scattering and frequency doubling scattering greatly. At the same time, the scattering intensities were proportional to the concentration of protein. Based on this, a new and fast spectral method with high sensitivity, good selectivity and simplicity for the determination of trace protein had been established. The proposed method was successfully applied to the determination of trace amount of proteins in synthesized samples, serum and urine samples with satisfactory results.

TABLE-2
EFFECT OF COEXISTING SUBSTANCES ON THE DETERMINATION OF 2 μg mL^{-1} BOVINE SERUM ALBUMIN

Foreign substance	Concentration	Change of I_{RRS} (%)	Foreign substance	Concentration	Change of I_{RRS} (%)
Mn(II)	5.0×10^{-5} mol L^{-1}	4.28	L-Arg	30 mg L^{-1}	4.4
Ca(II)	5.0×10^{-5} mol L^{-1}	3.80	L-Leu	40 mg L^{-1}	-3.8
Ni(II)	5.0×10^{-5} mol L^{-1}	-4.9	L-Cys	25 mg L^{-1}	-4.5
Mg(II)	5.0×10^{-5} mol L^{-1}	5.2	L-Tyr	15 mg L^{-1}	-4.6
Co(II)	4.0×10^{-5} mol L^{-1}	-2.9	L-Lys	20 mg L^{-1}	-4.3
Cd(II)	3.0×10^{-5} mol L^{-1}	4.73	L-Ser	30 mg L^{-1}	4.9
Zn(II)	3.0×10^{-5} mol L^{-1}	-2.7	L-Try	25 mg L^{-1}	3.8
Fe(II)	1.0×10^{-5} mol L^{-1}	4.98	L-Pro	30 mg L^{-1}	-4.7
Cr(II)	4.0×10^{-6} mol L^{-1}	4.02	L-Glu	20 mg L^{-1}	-1.8
Hg(II)	1.0×10^{-6} mol L^{-1}	2.38	L-His	40 mg L^{-1}	4.8
Pb(II)	1.0×10^{-6} mol L^{-1}	3.72	L-Val	20 mg L^{-1}	3.1
Citrate	1.0×10^{-3} mol L^{-1}	2.1	Glucose	60 mg L^{-1}	-2.79

TABLE.-3
RESULTS OF THE DETERMINATION OF PROTEIN IN SYNTHESIZED SAMPLES USING THE RESONANCE RAYLEIGH SCATTERING METHOD

Sample	Bovine serum albumin added (μg mL^{-1})	Main additives (μg mL^{-1})	Found value (μg mL^{-1})	RSD (%)	Recovery (n = 5 %)
No.1	2.0	K^+, Cl^- 2.0; Al^{3+}, SO_4^{2-} 2.0; Fe^{3+}, SO_4^{2-} 2.0; CO^{2+}, Ac^- 2.0; Ni^{2+}, SO_4^{2-} 0.5; Mg^{2+}, SO_4^{2-} 2.0; D-fructose 0.5; L-Isoleucine 0.5; L-Phenylalanine 0.5;	1.94	1.4	97
No.2	2.0	Pb^{2+}, Ac^- 0.5; Na^+, CO_3^{2-} 5; Ba^{2+}, Cl^- 4; Na^+, SO_4^{2-} 2.0; NH_4^+, Ac^- 4; Zn^{2+}, SO_4^{2-} 2.0; Cu^{2+}, Cl^- 2; K^+, HCO_3^- 3; L-Tyrosin1e; L-Tyrosine 5	1.98	1.7	99
No.3	2.0	L-Cysteine 5; L-Leucine 5; L-Tyrosine 5; L- Lysine 5; glucose 5; Ca^{2+}, Cl^- 5; Na^+, SO_4^{2-} 2.0; K^+, HPO_4^- 3; Mn^{2+}, SO_4^{2+} 5	2.02	3.1	101
No.4	2.0	L-cystine 5; L-leucine 5; Na^+, CO_3^{2-} 10; Na^+, NO_2^- 6; Vitamin C 10; D-fructose 5	1.97	2.5	98.5

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