The Second-order Scattering of the Interaction of Pd Nanoparticles with Protein and Its Analytical Application

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The second-order scattering (SOS) phenomenon of the interaction of Pd nanoparticles with protein was reported and a simple, sensitive, palladium nanoparticle-based assay for trace amount of protein with SOS technique was developed. The SOS intensities were significantly enhanced due to the interaction of Pd nanoparticles with bovine serum albumin (BSA) or human serum albumin (HSA) at pH 3.5 or 4.0, respectively. The maximum SOS peak appeared at 260/520 nm ($\lambda_{ex}/\lambda_{em}$). The optimal experiment conditions, affecting factors and the influence of some coexisting substances were checked. The SOS intensity increased proportionally with the increase of Pd concentration below 3.0×10^{-5} mol·L^{-1}, while declined gradually above 4.0×10^{-5} mol·L^{-1}. BSA within the range of 0.01-2.6 μg·mL^{-1} and HSA of 0.01-1.7 μg·mL^{-1} can be detected with this method and the detection limits were 2.3 and 11.2 ng·mL^{-1}, respectively. The method was successfully applied to the quantitative detection of total protein content in human serum samples with the maximum relative standard deviation (RSD) lower than 2.6% and the recoveries over the range of 99.5-100.5%.

Key Words : Palladium nanoparticles, Second-order scattering, Protein detection, Bovine serum albumin

Introduction

As very important life substances, the quantitative detection and identification of trace amount of protein are required in biochemistry and molecular biology. Light scattering is a common phenomenon and has a wide application since Pasternack developed a resonance light scattering (RLS) technique by using a common spectrofluorometer in 1993.[1,2] As a basis of new technique, RLS has been applied to detection of biomolecules such as proteins and nucleic acids.[3,4] RLS is a particular synchronous scanning spectrum at $\Delta\lambda = 0.0$ nm ($\lambda_{ex} = \lambda_{em}$). Meanwhile, it is well known that many systems with strong RLS could produce strong light scattering at the double excitation wavelength ($2\lambda_{ex}$), which was named as second-order scattering (SOS).[5] However, SOS was regarded as an interfering peak and always eliminated in the fluorometric detection. SOS peak is an emission peak that sites at the double exciting wavelength ($\lambda_{em} = 2\lambda_{ex}$) and was first investigated as an analytical technique in 1995.[6] Presently SOS was extensively applied in detecting trace amounts of inorganic ions,[7-9] cation surfactants,[10] β-cyclodextrin[11] and nucleic acids.[12,13]

In recent years, there has been an increasing interest in using noble metal nanoparticles in biomolecules detection due to numerous advantages with these probes compared to those conventional detection methods, which are based on organic dyes, such as the Lowry,[14] Coomassie brilliant blue (CBB),[15] bromophenol blue[16] and bromocresol green[17] methods. Metal nanoparticles can overcome many of the significant chemical and spectral limitations of organic dyes.[18] Therefore, various metal nanoparticles-based approaches have been reported for protein detection, such as spectrophotometric,[19] spectrofluorimetric,[20] chemiluminescence,[21] electrochemical[22] methods. There were some reports about RLS technique for proteins detection employing metal nanoparticles,[23-25] but there were fewer studies on metal nanoparticle-based SOS methods for proteins detection. In this paper, a new method for the detection of proteins with SOS technique based on the significant enhancement of SOS signals due to the interaction of Pd nanoparticles with protein molecules was developed, and the SOS characteristics and the optimal experiment conditions as well as affecting factors were investigated. The proposed method was applied to the detection of total protein contents in human serum samples with satisfying results.

Experimental Section

Reagents. Palladium chloride ($PdCl_2$), sodium citrate and other chemicals were of analytical grade. $H_2PdCl_4 \cdot nH_2O$ was prepared by treating $PdCl_2$ with concentrated hydrochloric acid. Stock solutions of 100 μg·mL^{-1} of bovine serum albumin (BSA) and human serum albumin (HSA) (Sigma, St. Louis, USA) were prepared and stored at 0-4 °C. Doubly distilled water was used throughout. Britton-Robinson (BR) buffer solution[26] was used to control the acidity of the tested solutions. Human serum samples were offered by the People's Hospital of Hubei Province and diluted to 25,000-fold volume with doubly distilled water before used.

Apparatus. Transmission electron microscopy (TEM) was measured with a Tecnai G2 20 electron microscope (FEI Company), operating at 300 KV. Samples for TEM measurements were prepared by placing a drop of the metal colloidal dispersion on a copper grid coated with a perforated carbon

film, followed by evaporation at room temperature. The particle diameters were measured from the enlarged photographs. The particle size distribution histogram was obtained on the basis of the measurements of about 300 particles. X-ray powder diffraction (XRD) was performed on a D8 Advance Bruker X-ray diffractometer. SOS spectra were recorded on a LS-55 fluorescence spectrometer. UV-vis spectra were measured on a Lambda BIO35 spectrophotometer. pH values were measured with a model of PHS-25 pH-meter (Shanghai, China).

Preparation of Pd nanoparticles. The preparation was carried out with the modified microwave oven. In a 100 mL flask, a solution of H_2PdCl_4 and 1% sodium citrate were added with stirring and a pale yellow solution was obtained. Then, the flask was put in the cavity of microwave oven (Galanz, 900 W, 2450 GHz). The mixture was heated for 60 s by microwave irradiation and then cooled fast with ice-salt bath. A transparent dark brown homogenous solution of colloidal Pd nanoparticles was obtained without any precipitate. Particle sizes of Pd colloids were measured by TEM prior to use.

Standard Procedure. 0.5 mL of 0.1 mmol·L^{-1} of Pd colloid, 1.0 mL of 1% NaCl solution, 1.0 mL of 0.05 mol·L^{-1} of BR buffer and 0.1 mL of 100 μg·mL^{-1} of BSA were added into a 10 mL volumetric flask in order and mixed thoroughly, and then made up to volume with doubly distilled water. The SOS intensities were measured with the excitation (λ_{ex}) and the emission wavelength ($\lambda_{em} = 2\lambda_{ex}$) by the spectrofluorometer. The SOS spectra were obtained by plotting the different wavelength against the SOS intensity, I_{sos}. The relative SOS intensities were measured at $\lambda_{ex}/\lambda_{em}$ = 260 nm/520 nm against the reagent blank which was prepared by the same way, but without any Pd or protein, and thus $\Delta I = I_{sos} - I^{\circ}_{sos}$ was obtained.

Results and Discussion

TEM images. A typical TEM micrograph of the Pd colloid without BSA is shown in Figure 1(a), while that with BSA is shown in Figure 1(b). As can be seen in the Figure, all the particles are well-dispersed. The average diameters of nanoparticles were 1.4 nm for neat Pd colloid and 2.4 nm for the Pd-BSA system and the corresponding standard deviations were 0.4 nm and 0.8 nm, respectively, as shown in Figure 1(c) and (d). A slightly larger particle diameter as well as wider size distribution for Pd colloid after mixing with BSA were observed, which showed that Pd nanoparticles interacted with BSA molecules and the interaction resulted in the formation of Pd-BSA complex.

SOS spectral characteristics. The SOS spectra of the BSA solution, neat Pd colloid and Pd-BSA complex system under optimum conditions are shown in Figure 2. It can be seen that both BSA solution and neat Pd colloid showed weak SOS signals, though four minor peaks at 242, 260, 298

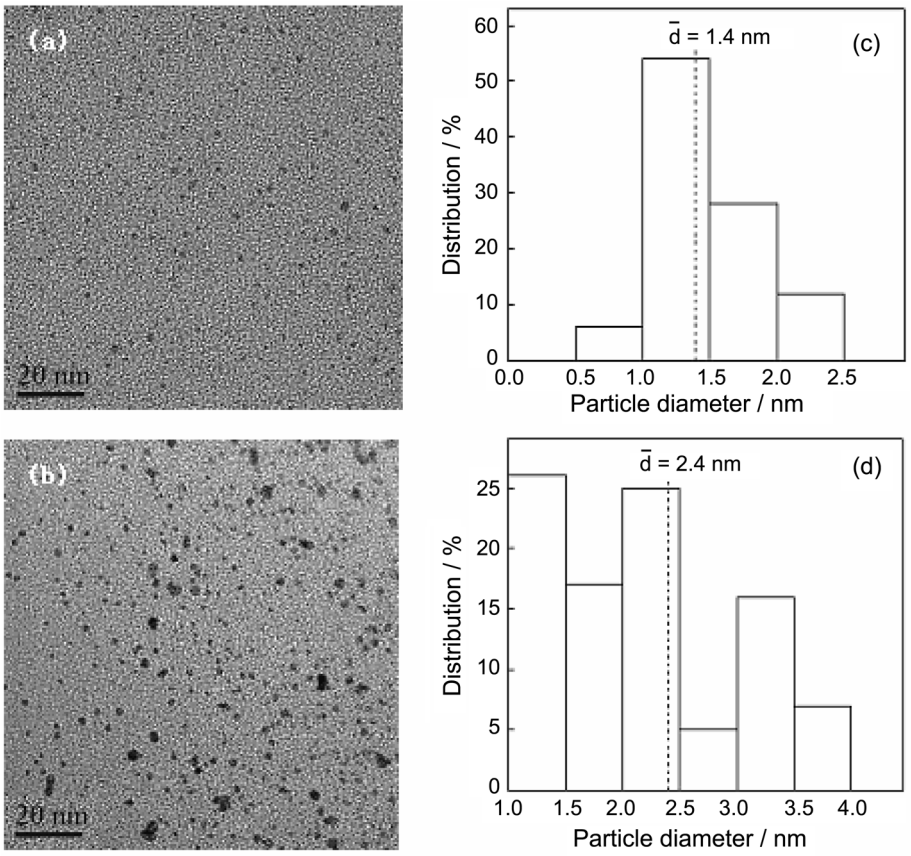

Figure 1. TEM micrographs of Pd colloids (a) without BSA; (b) with BSA; and the corresponding particle size distribution histograms (c) and (d), respectively. Scale bars: 20 nm.

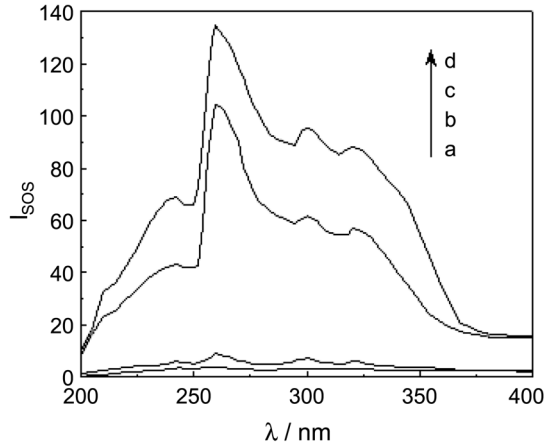

Figure 2. SOS spectra under the optimal conditions. (a) BSA solution only; (b) neat Pd colloids; (c) and (d) Pd colloids with 1 $\mu g \cdot mL^{-1}$ and 2 $\mu g \cdot mL^{-1}$ of BSA, respectively. Pd concentration was 4.0×10^{-5} mol·L^{-1}.

and 320 nm were observed for neat colloidal Pd nanoparticles. However, the SOS intensity was remarkably enhanced when adding BSA into the Pd colloid, indicating the formation of Pd-BSA complex due to the interaction of Pd nanoparticles with BSA molecules. The maximum SOS peak is at 260/520 nm, and the SOS intensity increased linearly with the increase of the content of BSA while keeping an appropriate concentration of Pd nanoparticles. A similar characteristic was also observed for HSA at the same condition. Based on these phenomena a method of quantitative detection of protein content can be established.

Effect of pH. Figure 3 shows the effect of pH values on the SOS intensities of the complex systems. It can be seen that pH had a great influence on the SOS intensities for Pd-BSA and Pd-HSA in the range of pH 2.0-10.0, and the optimum pH values were obtained at pH 3.5 and 4.0, respectively, at which the ΔI values of the corresponding complexes reached the maximum. At the optimum pH values, which are lower than the isoelectric point of BSA or HSA (pI = 4.7-4.9), the surface of the peptide molecules

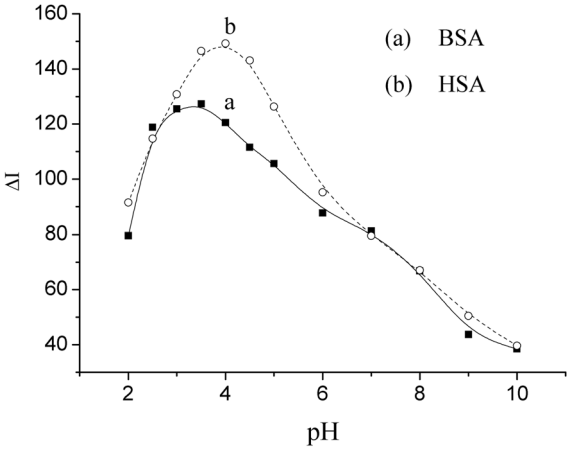

Figure 3. Effect of pH on the enhancement of SOS intensities. (a) Pd-BSA complex; (b) Pd-HSA complex; Pd concentration was 4.0×10^{-5} mol·L^{-1}; The concentration of BSA or HSA was 2.0 $\mu g \cdot mL^{-1}$.

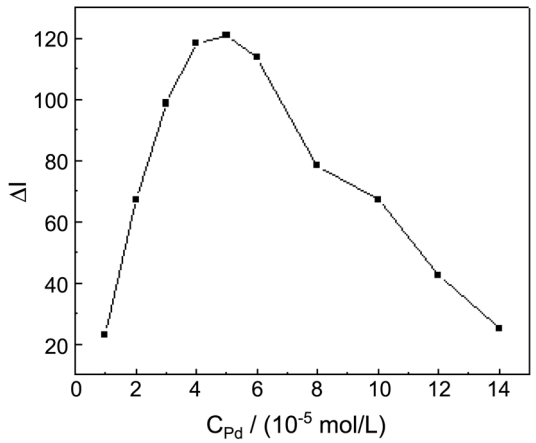

Figure 4. Effect of the concentration of Pd nanoparticles on enhancement of SOS intensity. $C_{BSA} = 2.0$ $\mu g \cdot mL^{-1}$; pH = 3.50.

hold positive charges due to the protonation of the amino groups of the basic amino acids of BSA or HSA. When protein was added into the Pd colloids, the electrostatic attraction and hydrophobic association resulted in an interaction between Pd nanoparticles and protein molecules. The interaction made Pd nanoparticles aggregated on the protein molecules to produce Pd-protein complexes and enhanced the SOS intensity. However, under a higher or lower pH, the ΔI reduced significantly due to strong repulsion or protein denaturation. In addition, the systems were buffered with 0.5-1.5 mL BR buffer solution to control the optimum pH in the present experiments and it gave good sensitivity as well as high stability. Since the volume of the added buffer had little influences on the SOS intensity, 1.0 mL of BR buffer was used in all subsequent experiments.

Effect of the concentration of Pd nanoparticles. It is well known that the number of the nanoparticles per unit volume is associated with the aggregation degree. The SOS intensity is dependent on the aggregation number of nanoparticles. The effects of Pd concentration on the SOS intensity are shown in Figure 4. It was found that the most appropriate concentration of Pd nanoparticles was 3.0×10^{-5}-4.0×10^{-5} mol·L^{-1} when the concentration of BSA was 2.0 $\mu g \cdot mL^{-1}$ at pH 3.5. Nevertheless, when Pd concentration was lower than 3.0×10^{-5} mol·L^{-1}, the SOS intensity and sensitivity reduced with the decrease of Pd concentration. The reason may be that the incomplete interaction of Pd with protein generated a lower aggregation number and smaller aggregates at a lower Pd concentration, resulting in less enhancement of SOS intensity. In other words, the SOS intensities enhanced gradually due to aggregation improving with an increase of Pd concentration up to 3.0×10^{-5} mol·L^{-1}. However, the SOS intensities declined gradually when Pd concentration was more than 4.0×10^{-5} mol·L^{-1}. The reason is that a higher concentration or large excess of Pd particles means relatively fewer protein molecules adsorbed on Pd nanoparticles, thus greatly reduced the aggregation number of protein with Pd particles per unit volume, resulting in a decrease of SOS intensity.

Effect of ionic strength. Ionic strength should have a little effect on the interaction of Pd nanoparticles with protein molecules. The experiments showed that the SOS remained a high sensitivity and intensity below 0.3% NaCl of ionic strength, while an obvious decline in the SOS signals was observed above 0.3% NaCl of ionic strength. So only 0.1% NaCl was used in all of the experiments.

Mixing sequence of reagents. The effect of the mixing sequence of Pd colloids, BSA, NaCl and BR buffer on SOS intensities was investigated. Though Pd nanoparticles interacted with protein rapidly at room temperature, the mixing sequence was very important. It was found that the favorable formation of Pd-protein complex was accomplished with the mixing sequence of Pd, BR buffer, NaCl solution and BSA. It is necessary for the interaction of Pd nanoparticles with protein to control the optimum pH and appropriate ionic strength, so the buffer and NaCl should be mixed with Pd nanoparticles before adding protein.

Interferences of coexisting substances on SOS. In order to evaluate the applicability of the method, the possible interferences of some coexisting substances such as metal ions, sugar, amino acids and proteins were investigated at 1.0 $\mu g \cdot mL^{-1}$ of BSA under the optimal conditions (allowable deviation ± 5%). The data are listed in Table 1. The results demonstrated that few of metal ions and amino acids interfered with protein detection except for several ions such as Ag^{+}, Cu^{2+}, Pb^{2+}. Possibly, the heavy metal ions can strongly complex with the amino groups and then interfere the detection to some extent. In addition, Hb has certain interference. In fact, the experimental concentrations of all these substances were much higher than the concentrations present in biological fluids. Furthermore, dilution with water can minimize all interferences in the detection of biological fluids such as human serum samples.

Calibration curves and detection limit. Under the optimal experiment conditions, the ΔI of the Pd-protein complexes were measured. A good linear relationship between ΔI and the content of protein was obtained. The correlation coefficient, linear regression equation, linear range and detection limit are given in Table 2. The results demonstrated that this method had a higher sensitivity and could be applicable in protein detection.

Table 1. Interferences of some coexisting substances on the determination of BSA

Substances	content ($\mu g \cdot mL^{-1}$)	deviation (%)	Substances	content ($\mu g \cdot mL^{-1}$)	deviation (%)
Cr^{3+}	10	0.8	Ag^{+}	5	8.5
Fe^{3+}	10	0.7	Urea	20	0.3
Ca^{2+}	20	0.16	Glucose	40	1.0
Zn^{2+}	5	–0.39	Lys	20	2.1
Ni^{2+}	1	0.3	Glu	10	2.6
Cu^{2+}	5	5.3	Phe	20	0.7
Sn^{2+}	5	3.0	Pro	20	3.4
Co^{2+}	10	1.0	Cys	20	–0.6
Pb^{2+}	2	3.3	His	20	2.6
Mn^{2+}	10	0.4	Hb	10	5.5

Table 2. The calibration curves of two kinds of serum albumins

protein	linear range ($\mu g \cdot mL^{-1}$)	linear regression equation ($\mu g \cdot mL^{-1}$)	detection limit ($ng \cdot mL^{-1}$)	correlation coefficient (R)
BSA	0.01-2.6	ΔI = 64.6 + 30.5 C	2.3	0.998
HSA	0.01-1.7	ΔI = 5.9 + 42.6C	11.2	0.999

Each result was the average of five measurements.

Table 3. Total content of proteins in human serum samples

Samples	SOS method ($mg \cdot mL^{-1}$)	CBB G-250 method ($mg \cdot mL^{-1}$)	recovery yield (%)	RSD (%)
1	74.3	74.0	99.5	2.1
2	73.6	73.5	99.9	2.5
3	75.1	75.5	100.5	2.6

Each result was the average of five measurements.

Detection of the total content of proteins in human serum samples. According to the general procedure described above, the total protein contents in human serum samples were determined by using the present method and HSA was chosen as the standard. The assaying data are presented in Table 3. The maximum relative standard deviation (RSD) was 2.6% and the recovery yields were about 99.5-100.5%. The total protein contents measured by the proposed method were in excellent agreement with those obtained by CBB method,[15] indicating that the present method can be used in total protein contents in human serum samples.

Conclusions

The method proposed here for protein detection is based on the significant enhancement of SOS signals due to the interaction of Pd nanoparticles with BSA molecules. Under the optimal conditions, the interaction of Pd nanoparticles with BSA demonstrated much strong SOS intensity in contrast with the weak SOS signal of neat Pd colloid or BSA alone. The enhanced SOS intensity implied the formation of Pd-BSA complex due to this interaction. Considering the experimental results, a highly sensitive palladium nanoparticles-based assay for protein detection with SOS technique was developed. The present method was satisfactorily applied to the detection of total protein contents in human serum samples and the results were very close to those obtained by conventional method. Therefore, the colloidal Pd nanoparticles-based assay with SOS technique may be promising for protein detection in biological samples.

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