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Resonance light scattering spectral method for the determination of serum albumin with the interaction of neutral red–sodium dodecyl sulfonate

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ABSTRACT

Based on the enhancement of resonance light scattering (RLS) of serum albumin interaction with neutral red (NR) and sodium dodecyl sulfonate (SDS), a novel sensitive assay of serum albumins has been developed. Experimental conditions such as mixing sequence of reagents, pH, NR and SDS concentrations have been optimized. Linear relationships between the enhanced RLS intensity and the protein concentration were observed for bovine serum albumin (BSA) within the range of 0.01–5.0 μg mL^{-1} and human serum albumin (HAS) of 0.01–7.0 μg mL^{-1}. The detection limits (S/N = 3) are 6.0 ng mL^{-1} for BSA and 5.0 ng mL^{-1} for HAS, respectively. The method was successfully applied to the determination of HSA in human blood plasma samples with recovery from 97.3 to 104.3%.

1. Introduction

The quantitative determination of serum albumins and its interaction with some small molecules are very important in clinical tests and laboratory practice since they are often used as references for measurement of other parameters in biological samples [1]. Among these, organic dyes have attracted great interest. Many analytical methods have been established for proteins assays based on the interaction of proteins with organic dyes. Among well known protein essays are the Lowry [2], Bradford [3,4], Bromophenol Blue [5] and Bromocresol Green [6] methods. However, most of these methods suffer from the disadvantages of low sensitivity [7].

Based on the aggregation of dyes in an aqueous medium with an incident light beam close to the absorption band of the aggregates, Pasternack et al. established the resonance light scattering (RLS) technique [8,9]. Henceforth, the RLS technique has received a great deal of extensive and intensive studies. It has been demonstrated that the enhanced RLS signals resulting from the binding of dye to nucleic acids or from the formation of ion associates can be used for the sensitive analysis of nucleic acids [10–13] and inorganic substances [14].

The aim of our current work is to develop a novel RLS technique to determine proteins, and to investigate the effect of NR and SDS on its determination. Results indicated that highest sensitivity and selectivity were obtained for different proteins (BSA and HAS) in the presence of NR and SDS. Practical applications of the developed method were demonstrated by its utilization in the accurate determination of HAS in human serum samples.

2. Experimental

2.1. Apparatus and reagents

The RLS spectrum was recorded with a LS-55 Fluorescence spectrophotometer (PerkinElmer, USA) by scanning simultaneously the excitation and emission spectra from 250.0 to 600.0 nm with $\Delta\lambda = 0$ nm and a slit width of 5.0 nm. Absorption spectra were scanned with a Lambda B10 35 Spectrophotometer (PerkinElmer, USA). A PHS-3 pH meter (Shanghai Scientific Instruments Company, China) was used to measure the pH values of the solutions.

Sodium dodecyl sulfonate and neutral red were both purchased from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China) and dissolved into water to obtain 1.0×10^{-3} and 1.0×10^{-4} mol L^{-1} solution respectively. Proteins including BSA (Sigma, USA) and HAS (B.M., Switzerland) were used as received. The proteins were directly dissolved in doubly distilled water to prepare stock solutions of 1.0 mg mL^{-1} and stored at 4 °C. Buffer solutions with different pH values were prepared by mixing 0.1 mol L^{-1} of sodium citrate and 0.1 mol L^{-1} of HCl in suitable proportions and the pH values were measured by a pH meter. Other reagents were of analytical reagent grade and doubly distilled water was used throughout.

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2.2. Experimental procedure

Into a 10-mL volumetric flask, 1.0 mL of pH 3.0 sodium citrate buffer solution, 1.0 mL of 1.0×1.0^{-3} mol L^{-1} SDS solution, appropriate amounts of working proteins such as HSA, BSA, and 0.3 mL of 1.0×10^{-4} mol L^{-1} neutral red solution were added in turn. The resulting solution was mixed thoroughly. The mixture was finally diluted to the mark using doubly distilled water and the flask was inverted 20 times to ensure complete mixing. It was then allowed to stand for 5 min, and the RLS spectra of the solutions were recorded with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ (i.e., $\Delta\lambda = 0$ nm). The RLS intensity was measured with the maximum wavelength at 363.0 nm. The RLS intensity increment of protein-NR-SDS system was represented as $\Delta I = I - I_0$, where I and I_0 were the RLS intensities of the NR-SDS system with and without serum albumin.

3. Results and discussions

3.1. Resonance light scattering spectral characteristics

Fig. 1 shows the RLS spectra of SDS, NR, BSA and their mixtures. As presented from curve 1 to 5, SDS, NR, BSA, SDS–NR, NR–BSA have rather weak RLS signals over the whole wavelength range of 250–600 nm. In the case of SDS–BSA mixture, the RLS intensity increased slightly. However, strong RLS signal was observed in the mixtures of SDS–NR–BSA with a maximum at 363.0 nm. In addition, the RLS intensity increased with increasing BSA concentration from 3 to 5 μg mL^{-1} (curves 7 and 8), indicating that an interaction has occurred between SDS, NR and BSA.

Weak absorption band at ~545 nm was observed for SDS–NR, NR and SDS as curves 1, 2 and 3 shown in Fig. 2. When BSA was added separately, a weak hypochromic effect was observed for the maximum absorption wavelength of NR–BSA and SDS–BSA system. No obvious change was observed in the case of NR–SDS–BSA (curve 6) and BSA (curve 7). Compared with the absorption peak of BSA, only slight increase in the absorption intensity is observed for NR–SDS–BSA system. This increase cannot be employed for the sensitive determination of BSA, whereas the strongly enhanced RLS signal (curve 8 in Fig. 1) allows BSA determination with a high sensitivity. HAS displays similar RLS-enhancing interactions with SDS–NR.

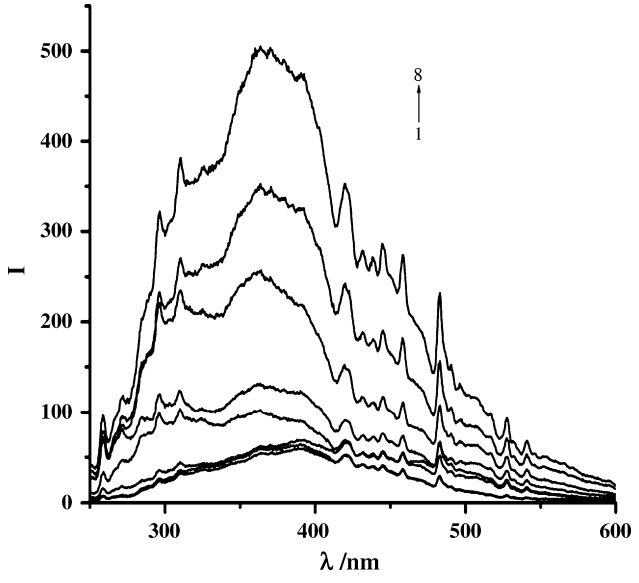

Fig. 1. Resonance light-scattering spectra of NR (curve 1), BSA (curve 2), NR–BSA (curve 3), SDS–NR (curve 4), SDS–BSA (curve 5) and SDS–NR–BSA mixture (curve 6). Concentrations: BSA 2.0 μg mL^{-1}, NR 3.0×10^{-6} mol L^{-1}, SDS 1.0×10^{-4} mol L^{-1}; pH 3.0. Curves 7–8 is the RLS spectra of SDS–NR–BSA mixture in presence of 3.0 and 5.0 μg mL^{-1} BSA.

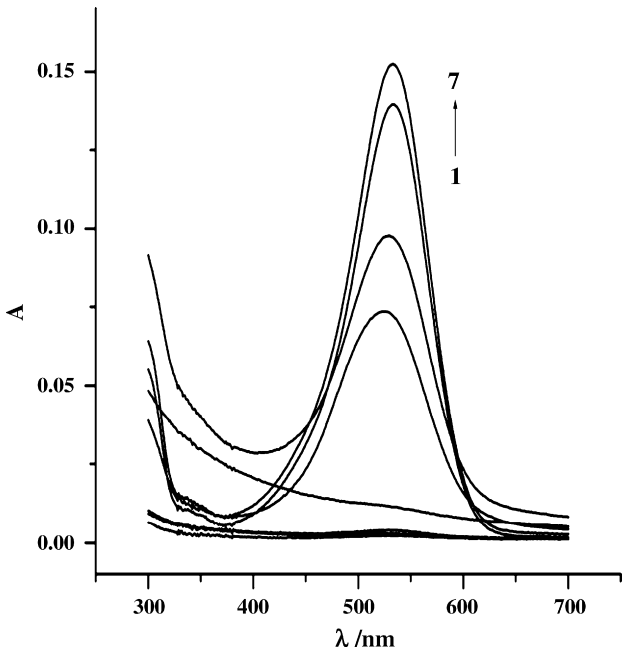

Fig. 2. Absorption spectra of SDS–NR (curve 1), NR (curve 2), SDS (curve 3), BSA–NR (curve 4), SDS–BSA (curve 5), SDS–BSA–NR (curve 6) and BSA (curve 7). Concentrations: BSA 5.0 μg mL^{-1}, NR 3.0×10^{-6} mol L^{-1}, SDS 1.0×10^{-4} mol L^{-1}; pH 3.0. The spectra were obtained against water.

3.2. Effect of pH

Fig. 3 shows the RLS intensity of SDS–NR system with (curve 1) and without (curve 2) BSA at different pH value. It is found that pH had a great influence on the RLS intensity for SDS–NR–BSA mixture in the range of pH 1.0–5.0 with the optimum pH values at pH 3.5, at which the ΔI values of the corresponding complexes reached the

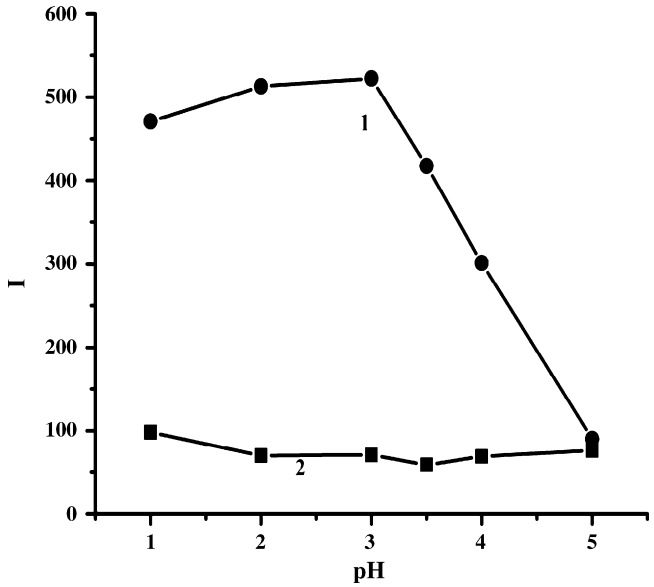

Fig. 3. Effect of pH on the RLS intensity of SDS–NR–BSA (curve 1) and NR–SDS (curve 2) mixtures. Concentrations: BSA 5.0 μg mL^{-1}, NR 3.0×10^{-6} mol L^{-1}, SDS 1.0×10^{-4} mol L^{-1}.