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Resonance light scattering technique for the determination of protein with rutin and cetylpyridine bromide system

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Abstract

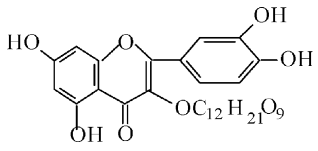
A new resonance light scattering (RLS) assay of protein is presented. In Tris–NaOH (pH = 10.93) buffer, the RLS of rutin–cetylpyridine bromide (CPB) system can be greatly enhanced by protein, including bovine serum albumin (BSA) and human serum albumin (HSA). The enhanced RLS intensities are in proportion to the concentration of proteins in the range of 5×10^{-9} to 2.5×10^{-6} g ml^{-1} for BSA and 2.5×10^{-8} to 3.5×10^{-6} g ml^{-1} for HSA. The detection limits (S/N = 3) are 3.0 ng ml^{-1} for BSA and 10.0 ng ml^{-1} for HSA. Samples are determined satisfactorily.

Keywords: RLS; Rutin; Protein; CPB

1. Introduction

The quantitative determination of proteins is very important in biochemical studies and biological techniques. The traditional methods are Branford method [1,2] and Lowry method [3]. Some spectrophotometric methods [4–7], fluorometric methods [8–10] and chemiluminescence methods [11–13] are also developed. Recently, the resonance light scattering (RLS) technique has become a new interesting method for determination of micro amounts of biomacromolecules. The technique is noted for its high sensitivity, simplicity and quickness. The determination is mainly based on the fact that the aggregation of dye chromophore on the biological macromolecule can give rise to strong RLS. Huang et al. [14] firstly used RLS technique to establish a new sensitive spectrophotometric method for DNA determination. Later on, this technique has been used for the determination of protein [15–19]. In this paper, a new method for protein assay by RLS with rutin and cationic surfactant CPB is developed.

Rutin is a compound of the Vitamin C family and has many physiological activities. It can maintain the resistance of the blood vessels, reduce their osmose and brittleness. In addition, rutin has many other functions, such as anti-inflammatory, reducing blood-fat, etc. Therefore, it has been used in clinical treatment [20]. The structure of rutin is as follows:



It is found that the RLS intensity of rutin can be greatly enhanced by protein (such as BSA and HSA) and CPB. The enhanced intensity is in proportion to the concentration of proteins in the range of 5×10^{-9} to 2.5×10^{-6} g ml^{-1} for BSA 2.5×10^{-8} to 3.5×10^{-6} g ml^{-1} for HSA. The detection limits are 3.0 ng ml^{-1} for BSA and 10.0 ng ml^{-1} for

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HSA. This method is a simple, sensitive technique for the determination of microgram amounts of proteins. The reaction mechanism has been investigated.

2. Experimental

2.1. Chemicals

Stock solutions of protein (100 μg ml^{-1}) were prepared by dissolving 0.0100 g of commercial BSA (Beijing Shuangxuan biological culture medium plant), HSA (Institute of Biomanufact, Shanghai) in 100ml volumetric flask with water. Both BSA and HSA are electrophoretic purity approximately 98%. According the absorption of BSA at 280.0 nm and its molar absorptivity, 1.0 μg ml^{-1} BSA is calculated to be 5.0 μmol l^{-1} BSA. These stock solutions needed to be stored at 0–4 °C and only occasionally gentle shake if needed.

A stock solution of cationic surfactant (CPB, 1.0 × 10^{-2} mol l^{-1}) was prepared by dissolving 0.9613 g CPB in 250 ml volumetric flask with water. Stock solution of rutin (5.0 × 10^{-3} mol l^{-1}) was prepared by dissolving 0.305 g rutin (collator for drug examination, the biological Product Examining Institute of the Medical Department of China) in anhydrous ethanol, then diluting to 100 ml with anhydrous ethanol. The solution was stored in a 100 ml brown measuring flask.

A 0.05 mol l^{-1} Tris–NaOH buffer was prepared by dissolving 3.03 g of Tris in 500 ml water, and adjusting the pH to 10.93 with NaOH.

All the chemicals used are of analytical grade and doubly deionized distilled water was used throughout.

2.2. Apparatus

The spectrum and intensity of RLS were measured with a Hitachi 850 spectrofluorimeter. All absorbance spectra are recorded with an UV-240 spectrophotometer (Shimadzu). All pH measurements were made with a pHS-2 acidity meter (Leici, Shanghai).

2.3. Experimental procedure

Into a 10 ml volumetric flask is successively added 0.5 ml of 1.0 × 10^{-3} mol l^{-1} CPB, 1.0 ml of Tris–NaOH buffer, appropriate amount of protein, 0.3 ml of 5.0 × 10^{-4} mol l^{-1} rutin. The mixture is diluted to mark with water and mixed thoroughly, then measure without additional incubation time. The RLS spectra is obtained by scanning simultaneously the excitation and emission monochromators from 250 to 600 nm with $\Delta\lambda$ = 0 nm. The intensity of RLS is measured at 460 nm in a 1 cm quartz cell with slit width at 10.0 nm for the excitation and emission. The enhanced RLS intensity of rutin–CPB–protein system is represented as $\Delta I_{RLS} = I_{RLS} - I^0_{RLS}$, here I_{RLS} and I^0_{RLS} are the intensity of the system with and without proteins.

3. Results and discussion

3.1. Spectra of resonance light scattering and absorption

Fig. 1 shows the light scattering spectra of rutin–BSA (a), CPB–BSA (b), rutin–BSA (c) and rutin–BSA–CPB (d). It can be seen from the RLS curve (c) and (d) that rutin–CPB and rutin–BSA–CPB systems have two RLS peaks at the wavelength of 350.0 and 460.0 nm, which are greatly enhanced by the addition of protein. From the absorption spectrum of rutin in Fig. 1 (curve (e)), it can be seen that rutin has two absorption peaks at 266.0 and 396.0 nm, respectively. By comparing curves d and e, it can be seen that the RLS peak lies at the red side of the absorption peak of rutin. According to the light scattering theory [21–25], this phenomenon can be explained as that the RLS peak is ascribed to the absorption of rutin. The addition of protein can cause stronger enhancement of ΔI_{RLS} at the wavelength of 460.0 nm than that at 350.0. So $\lambda_{em} = \lambda_{ex}$ = 460.0 nm is selected for further research.

3.2. Optimization of general procedure

3.2.1. Effect of pH and buffers

The effect of pH is shown in Fig. 2. It can be seen that pH cannot only influence I^0_{RLS}, but also influence I_{RLS} and ΔI_{RLS}. But the enhancement for ΔI_{RLS} and I_{RLS} is greater than that for I^0_{RLS}, and the maximum ΔI_{RLS} is obtained at pH = 10.93. Experimental results prove that different kind of buffer has different effect on ΔI_{RLS}. The ΔI_{RLS} (%) for Tris–NaOH, HMTA–HCL, NaAc–HAc, H_3BO_3–NaOH, NH_3–NH_4Cl is 100.0, 78.0, 62.1, 46.2, 38.7 respectively. The results indicate that ΔI_{RLS} is the largest in Tris–NaOH buffer, so the Tris–NaOH buffer is chosen for the assay and the optimum volume of buffer is 1.0 ml.

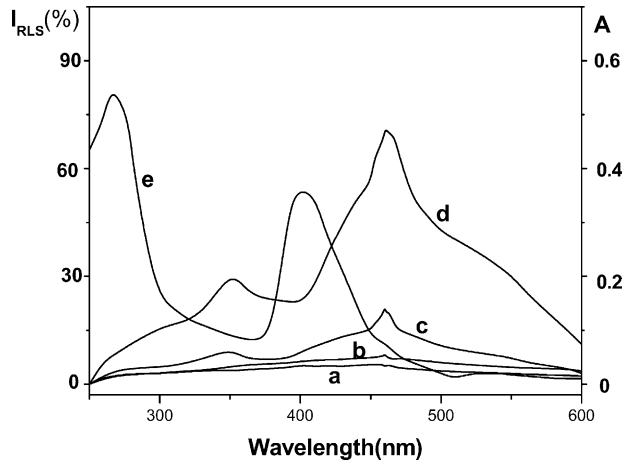

Fig. 1. Resonance light scattering spectrum (curve a–d) and absorption spectrum (curve e): (a) rutin–BSA; (b) CPB–BSA; (c) rutin–CPB; (d) rutin–BSA–CPB. Conditions: rutin, 1.5 × 10^{-5} mol l^{-1}; BSA, 5.0 × 10^{-6} g ml^{-1}; CPB, 5.0 × 10^{-6} mol l^{-1}, pH 10.93.