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Selective Determination of Nanogram γ–Globulin in Human Blood Serum by Resonance Light Scattering of Functionalized Nano–PbS

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Abstract: Functionalized nano-PbS has been prepared and characterized. The functionalized nanoparticles have good dispersibility in water. Reaction of functionalized nano–PbS with γ–globulin (γ–IgG) results an enhanced resonance light scattering (RLS) around 385 nm. However, when the content of HSA is lower than 0.5 μg ml^{-1} the RLS enhancement is very weak and is nonlinear to concentration of HSA. Based on these results, a new direct quantitative determination method for γ–globulin in blood serum samples without separation is established. Under optimal conditions, the enhanced RLS intensity is in proportion to the γ–IgG concentration in the range 10–500 ng/mL. The limit of detection is 2.75 ng/mL. This method is proved to be very sensitive, rapid, simple and selective for detection of γ–IgG in blood serum.

Keywords: Direct quantification, γ–globulin, resonance light scattering, functionalized nano–PbS.

The determination of proteins is a basic requisite in biochemistry because it is often used as a reference for the measurements of other components in biological systems. Resonance light scattering (RLS) is a valuable technique for quantification of proteins since the enhanced RLS signals can be easily measured by using a common spectrofluorometer for aggregated species or large particles in nanometer scale near UV absorption bands[1, 2]. These methods are sensitive and simple, but the selectivity of the direct quantification of γ–IgG is not satisfy.

In recent years, a new direction of biological surface modification of semiconductor [3, 4] and metal NPs[5, 6] with antibodies[3,4] and peptides[5, 6] received a lot of attention. Biomodified NPs from a variety of inorganic materials can be used in life sciences for luminescence tagging, drug delivery, and implantable microdevices as well as for assembling hybrid protein-NP units for molecular electronics. We have reported a class of nanometer-sized luminescent particles, which allow for the ultrasensitive detection of the concentration of nucleic acids[7]. In this paper, functionalized nanoparticles (PbS)-SCH_2COOH were prepared. They are highly resistant to photobleaching and show bright and steady resonance light scattering. The I_{RLS} of functionalized nano-PbS can be enhanced only by γ–globulin, and the resonance light scattering is proportional to the concentration of γ–globulin. So a new method for direct determination of gamma

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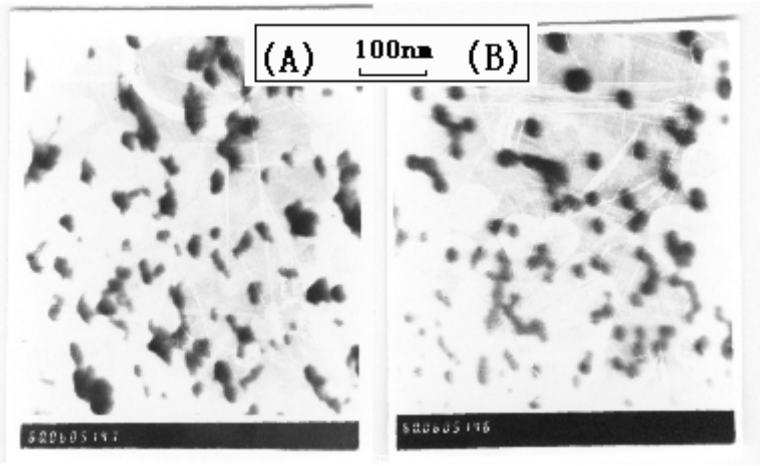
globulin in blood serum samples was established. The procedure is simplified and the method is very sensitive for gamma globuline.

The functionalized nano-PbS colloids were prepared as follows: Into a three-necked round-bottomed flask, 2 L of deionized water, 4.0 mL of 0.1 mol/L Pb $(NO_3)_2$ and 4.0 mL of 0.1 mol/L sodium hexametaphosphate as the precursors and the stabilizer, were added. Then the pH was adjusted to 9.0 with 0.1 mol/L NaOH solution. Under vigorous stirring and ultrasonic radiation, 4.0 mL of $Na_2S{\cdot}9\ H_2O$, was dropped slowly into the flask. Then the PbS colloids was concentrated to 50 mL by rotary evaporation. Under vigorous stirring ultrasonic radiatio, 8.0 mL of 1.0 mol/L mercaptoacetic acid solution was dropped slowly into the flask. The ultrasonic radiation was kept for 3 h. Excess mercaptoacetic acid was removed by repeated centrifugation[4, 6]. In the PbS nanoparticles, the mercapto group coordinated with Pb atom, and the polar carboxylic acid group made the nanoparticles easy to be dispersed in water, the free carboxyl group is also available for coupling with various biomolecules, such as proteins and peptides.

TEM image of functionalized PbS nanoparticles are shown in **Figure 1**. The diameter of the functionalized nano-PbS is about 23 nm. In addition, the TEM images showed that the diameter of nanoparticles increased by adding γ–globulin into the functionalized PbS colloidal solution. The IR spectrum, showed the peak at $\nu_{c=o}$ = 1655 cm^{-1}, characteristic for the mercaptoacetic acid capped onto the outer surface of the PbS nanoparticles.

Figure 1 TEM images of functionalized PbS nanoparticles in the presence (A) and absence (B) of gamma globulin



Under optimal conditions, the linear range is 10–500 ng/mL. The 3σ limit of detection is 2.75 ng/mL and the coefficient of correlation is 0.9975. The regression equation of this assay is I_{RLS} = 839.1 + 2.24 C (ng/mL). The human serum samples obtained from the No. 1 Renming Hospital, were diluted 1000-fold with deionized water before measurement. The results of the measurement with RLS technique were listed in **Table 1**. Comparing with the clinical data, the results are very satisfactory.

The main advantages of the enhanced RLS technique are higher sensitive, more

simple, reproducibility, and it is unaffected by amino acids, HSA, BSA, nucleic acids and most of the common ions in the serum samples. So this RLS technique can be expanded to the application in biochemistry and clinic.

Table 1 Analytical results for γ–globulin in human serum samples

Sample No.	Content of protein		Recovery (%, n = 6)	RSD (%)
	The clinical data (mg/mL) [b]	This method (mg/mL, n = 6)		
1	25.4	25.7	98 - 102	1.5
2	25.7	25.9	99 - 104	2.1
3	25.0	24.8	97 - 101	1.8

[a] Functionalized nano-PbS 3.0×10^{-4} mol/L; pH 7.0; [b] The data got from the hospital.

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References

1. L. J. Dong, R. P. Jia, Q. F. Li, X. G. Chen, Z. D. Hu, *Anal. Chim. Acta*, **2002**, *459*, 313.
2. C. Q. Ma, K. A. Li, S. Y. Tong, *Anal. Biochem.*, **1996**, *239*, 86.
3. M. Bruchez Jr., M. Moronne, P. Gin, S. Weiss, A. P. Alivisatos, *Science*, **1998,** *281*, 2013.
4. W. C. W. Chan, S. Nie, *Science*, **1998**, *281*, 2016.
5. C. A. Mirkin, R. L. Letsinger, R. C. Mucic, J. J. Storhoff, *Nature*, **1996**, *382*, 607.
6. J. J. Storhoff, R. Elghanian, R. C. Mucic, *et al.*, *J. Am. Chem. Soc.*, **1998**, *120*, 1959.
7. L. Y. Wang, L. Wang, F. Gao, Z. Y. Yu, Z. M. Wu, *Analyst*, **2002**, *127*, 977.

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