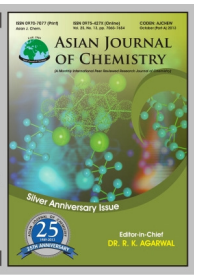
Asian Journal of Chemistry; Vol. 25, No. 15 (2013), 8769-8771

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15561



Quantitative Detection of Proteins by $[Co(NH_3)_6]^{3+}$-DNA Probe of Resonance Light Scattering Technique

Y.K. LI

College of Environment Science and Engineering, North China Electric Power University, Baoding, P.R. China

Corresponding author: Tel: +86 312 7522243; E-mail: 309267061@qq.com; lyk800@tom.com

(*Received*: 2 April 2013; *Accepted*: 29 August 2013) AJC-14045

$[Co(NH_3)_6]^{3+}$-DNA complex produces strong electrical force with proteins, which results in strong enhancement of resonance light scattering intensity. Resonance light scattering intensity at 346 nm was proportional to the concentration of bovine serum albumin in the range of 0.05-1.3 μg/mL. The limit of determination for bovine serum albumin has obtained at nanogram level. The method is applied to the assay of bovine serum albumin and good results have been obtained compared with custom measure methods. A sensitive, accurate and convenient new analysis method for micro-determination of proteins is established.

Key Words: Resonance light scattering, Proteins, Deoxyribonucleic acid, Hexaamminecobalt(III) ion.

INTRODUCTION

Protein is one of the most important materials in organisms and is the basis of life. Variety and content determination of protein is important for biochemical analysis and disease diagnosis. At present, the traditional method for protein determination by spectrophotometry method (including Kjeldahl method, biuret method and coomassie brilliant blue method) and fluorescence spectrophotometry[1-3]. The main disadvantage of spectrophotometry method is the relatively low sensitivity, so it cannot be used for the analysis of low protein concentration in the biological sample. Fluorescence spectrophotometry is limited to the fluorescent system, so the range of application is restricted. Therefore, the search for new protein detection method has important significance. Resonance light scattering (RLS) analysis method[4-6] is the light scattering analysis technology measured on a common fluorescence spectrophotometer. High sensitivity can be obtained as fluorescence method in research of biological molecular recognition, assembly and aggregation. Pasternack first used RLS technique to achieve the aggregation of porphyrins on nucleic acid. At present, this technique is widely used in quantitative detection of nucleic acids, proteins, polysaccharides and other biological macromolecules, as well as for interaction research of macromolecules, biological macromolecules and other substances.

The literature survey on the organic dye probes or metal ion indicator are mostly used in analysis of biological macromolecule by RLS spectra, but study on proteins analysis application by simple metal complexes of $[Co(NH_3)_6]^{3+}$-DNA-protein system in resonance light scattering technique has not been reported. In this assay, resonance light scattering signal of $[Co(NH_3)_6]^{3+}$-DNA-protein system can be strongly enhanced compared with proteins, $[Co(NH_3)_6]^{3+}$-proteins, DNA-proteins system. According to the above characteristics, in a certain protein concentration range, resonance light scattering signal enhancement and protein concentration has a linear relation.

EXPERIMENTAL

Hitachi F-4500 fluorescence spectrophotometer (Japan). A WH-861 voxtex mixer (Taicang Instrumental Co., JiangSu, China) was used to blend the solution. Stock solutions of nucleic acids (10 μg/mL) were prepared at 0-4 ºC by dissolving calf thymus DNA (ctDNA, Sigma D-1501) in water. Stock solutions of bovine serum albumin (BSA) (100 μg/mL) were prepared at 0-4 ºC by dissolving BSA in water. A 1 mmol/L hexaamminecobalt(III) chloride ($[Co(NH_3)_6]Cl_3$) solution was prepared. The stock solution of NaCl was prepared at 0.5 mol/L. Britton-Robinson (BR) buffers were applied for pH 1.8-9.0. All reagents were of analytical reagent grade and doubly deionized water was used throughout.

In a 10 mL volumetric flask, 0.7 mL 10 mg/mL ctDNA, 1.5 mL Britton-Robinson buffer (pH 3.78), 0.1 mL 1 mmol/L $[Co(NH_3)_6]Cl_3$ were introduced in turn and a certain volume of sample solution were transferred in. Finally, the mixture was diluted to 10 mL with deionized water and blended thoroughly. After incubation at room temperature for 1 h, RLS spectra were obtained with scanning simultaneously with the same excitation and emission wavelengths from 200-700 nm

by F-4500 fluorescence spectrophotometer and the RLS intensities were obtained at the wavelength of 346.0 nm. The slit width and PMT voltage of the measurements were 5 nm and 400 V, respectively.

RESULTS AND DISCUSSION

Light scattering spectra: Fig. 1 shows the resonance light scattering (RLS) spectrum of different proteins system. According to the resonance light scattering theory[7-10], particle volume increasing, the strong electrostatic binding and binding number leads to highly electron delocalization conjugate, which can produces intense resonance light scattering phenomenon. In the wavelength range of 200-700 nm, light scattering is strongly enhanced and reaches maximum at 346 nm.

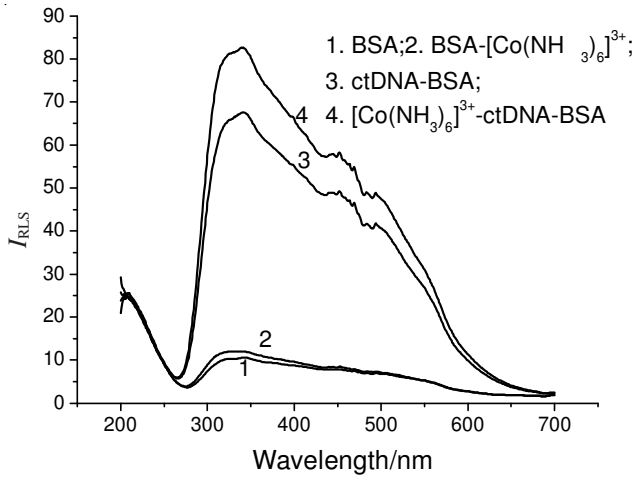


Fig. 1. Resonance light-scattering spectra; ctDNA (mg/mL): 0.7; $[Co(NH_3)_6]^{3+}$(mol/L): 10^{-5}; BSA (mg/mL): 1.0; pH = 3.78

Optimization of the general procedure

Influence of pH: Fig. 2 shows the influence of pH on the RLS intensity of $[Co(NH_3)_6]^{3+}$-DNA in the absence and presence of BSA. In pH 2.21-4.56, ctDNA-$[Co(NH_3)_6]^{3+}$-BSA produces strong RLS intensity enhancement, which is attributed to Phosphate ions (PO_4^{3-}) with negative charge distributed at the surface of DNA molecule. Protein isoelectric point is pH 4.7, so BSA is with positive charge in pH < 4.7[11]. The combination of BSA and DNA can produce strong RLS signal and the $[Co(NH_3)_6]^{3+}$ and DNA combining makes the DNA-BSA system signal increasing further, so test sensitivity is improved. In Fig. 2, the RLS intensity enhanced by BSA had a maximum value at pH 3.78.

Influence of the volume of Britton-Robinson buffer: Fig. 3 shows BR buffer (pH 3.78) 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 mL was, respectively added. It can be seen from Fig. 3, when BR buffer is lower than 1.5 mL, RLS intensity of $[Co(NH_3)_6]^{3+}$-DNA is comparably bigger and the differences between RLS intensity of $[Co(NH_3)_6]^{3+}$-DNA and $[Co(NH_3)_6]^{3+}$-DNA-BSA (ΔI) are comparably smaller. When BR buffer is higher than 1.5 mL, the both RLS intensities are almost constant. Finally, 1.5 mL BR buffer was chosen as the optimum volume.

Influence of the concentration of $[Co(NH_3)_6]^{3+}$: At pH 3.78, BR buffer, when the concentrations of DNA and BSA were settled, the effect of the concentration of $[Co(NH_3)_6]^{3+}$ on the RLS intensity was shown in Fig. 4. The concentration of $[Co(NH_3)_6]^{3+}$ was raised from 0 to 1×10^{-4} mol/L. It is shown that, when $[Co(NH_3)_6]^{3+}$ concentration is 10^{-5} mol/L, the difference between RLS intensity of $[Co(NH_3)_6]^{3+}$-DNA and $[Co(NH_3)_6]^{3+}$-DNA-BSA are comparably the highest. So the $[Co(NH_3)_6]^{3+}$ concentration at 10^{-5} mol/L was chosen for the assay.

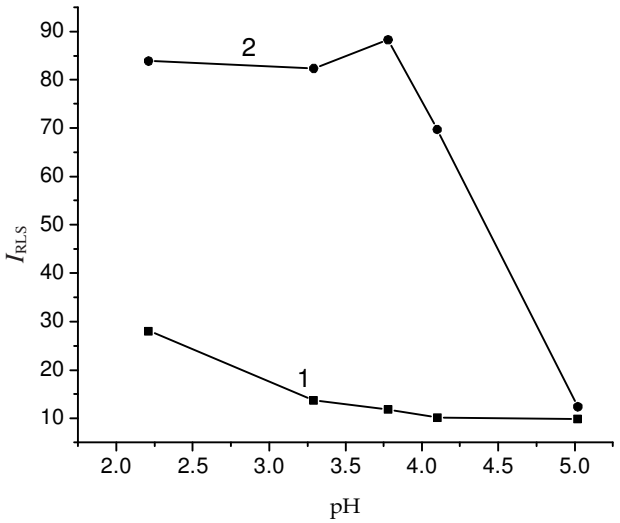


Fig. 2. RLS intensity with different pH values. 1. ctDNA-$[Co(NH_3)_6]^{3+}$; 2. ctDNA-$[Co(NH_3)_6]^{3+}$-BSA; ctDNA (mg/mL): 0.7; $[Co(NH_3)_6]^{3+}$(mol/L): 10^{-5}; BSA (mg/mL): 1

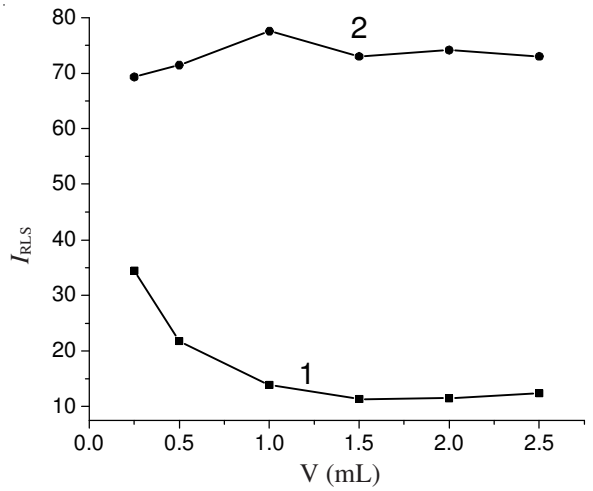


Fig. 3. RLS intensity with different volumes of BR solution. 1. ctDNA-$[Co(NH_3)_6]^{3+}$; 2. ctDNA-$[Co(NH_3)_6]^{3+}$-BSA; ctDNA (mg/mL): 0.7; $[Co(NH_3)_6]^{3+}$(mol/L): 10^{-5}; BSA(mg/mL): 1

Influence of the concentration of ctDNA: At pH 3.78 BR buffer, when the concentrations of $[Co(NH_3)_6]^{3+}$ and BSA were settled, the effect of the concentration of DNA on the RLS intensity was shown in Fig. 5. It is shown that, when concentration of ctDNA is higher than 0.7 mg/mL, RLS intensity of $[Co(NH_3)_6]^{3+}$-DNA-BSA are almost constant. So the ctDNA concentration at 0.7 mg/mL was chosen for the assay.

Calibration curves: According to above general procedure, the calibration curves for determination of BSA were constructed under the determined optimal conditions. As the Fig. 6 shows, there are good linear relationships between the

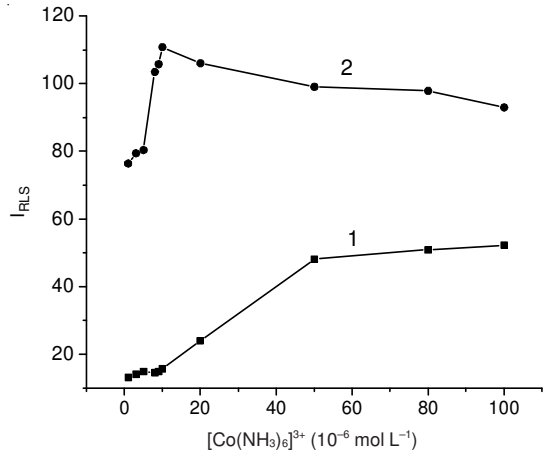


Fig. 4. RLS intensity with different $[Co(NH_3)_6]^{3+}$ concentration; 1. ctDNA-$[Co(NH_3)_6]^{3+}$; 2. ctDNA-$[Co(NH_3)_6]^{3+}$-BSA; ctDNA (mg/mL): 0.7; $[Co(NH_3)_6]^{3+}$(mol/L): 10^{-5}; BSA(mg/mL): 1.0

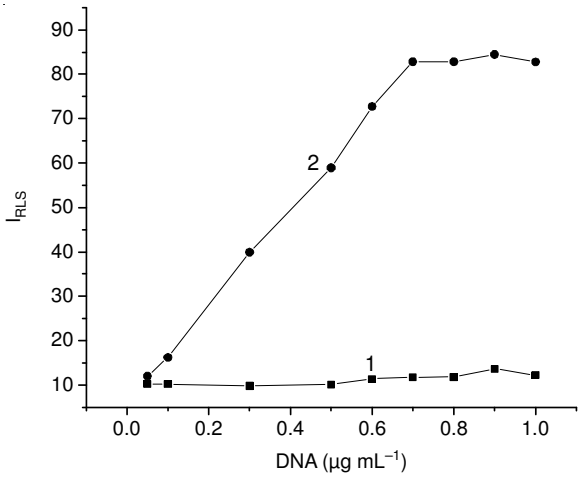


Fig. 5. RLS intensity with different DNA concentration; 1. ctDNA-$[Co(NH_3)_6]^{3+}$; 2. ctDNA-$[Co(NH_3)_6]^{3+}$-BSA; ctDNA (mg/mL): 0.7; $[Co(NH_3)_6]^{3+}$(mol/L): 10^{-5}; BSA(mg/mL): 1.0

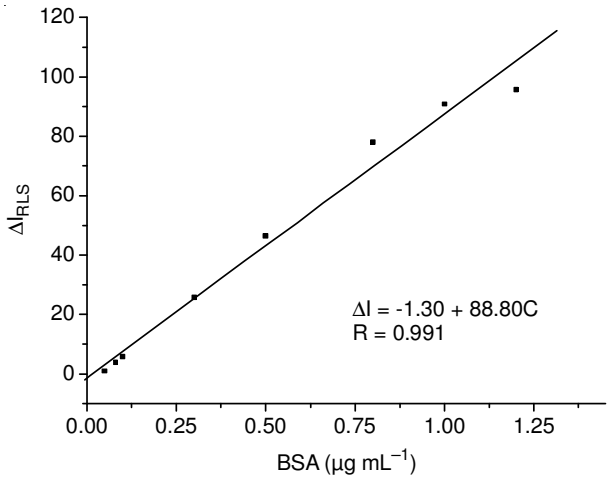


Fig. 6. Relationships between BSA concentrations and enhanced RLS intensity

enhanced RLS intensity (ΔI) and the concentration of BSA (C) over a wide range of 0.05-1.2 μg/mL and the sensitivity of the RLS assay was high.

Assay of bovine serum albumin samples: Two unknown sample solutions of BSA were measured by RLS method and Bradford (CBB G-250) method at the same time and the results were list in Table-1. According to the average recovery and relative standard derivation (RSD) of three measurements, RLS method for proteins assay is accurate and reliable.

TABLE-1
ASSAY OF BSA CONCENTRATION

Sample	C (μg/mL)[CBB G-250]	C (μg/mL) [RLS]	Recovery (%)	RSD (%) (n = 3)
1	0.80	0.77	96.3	2.5
2	0.44	0.42	95.5	1.8

Conclusion

By using $[Co(NH_3)_6]^{3+}$-DNA as a probe, the RLS technique for proteins determination has been proposed and successfully applied to the determination of BSA. It can be characterized with obvious advantages in respect of widely linear range and high sensitivity, simple operation procedures.

ACKNOWLEDGEMENTS

This study is supported by The National Natural Science Foundation of China (No. 21305043), the Natural Science Foundation of Hebei Province (No. B2011502061) and the Fundamental Research Funds for the Central Universities (No. 12MS105).

REFERENCES

1. H.M. Wang, P. Zhou, Y.Y. Long, H.B. Chen, Y.F. Yang, N. Li and F. Liu, *Talanta*, **216**, 94 (2012).
2. L. Zhan, L. Peng, Y. Yu, S.J. Zhen and C.Z. Huang, *Analyst*, **4968**, 137 (2012).
3. H.C. Kuo and J.H. Li, *J. Conv. Inf. Technol.*, **176**, 7 (2012).
4. C.X. Yang, Y.F. Li, P. Feng and C.Z. Huang, *Chin. J. Anal. Chem.*, **473**, 30 (2002).
5. C.Z. Huang and Y.F. Li, *Anal. Chim. Acta*, **105**, 500 (2003).
6. Q.L. Yue, T.F. Shen, J.T. Wang, L. Wang, S. L. Xu, H.B. Li and J.F. Liu, *Chem. Commun.*, **1750**, 49 (2013).
7. R.F. Pasternak and P.J. Collings, *Science*, **935**, 269 (1995).
8. R.F. Pasternak, C. Bustamante and P.J. Collings, *J. Am. Chem. Soc.*, **5393**, 115 (1993).
9. Z.G. Chen, S.H. Qian, X. Chen, J.H. Chen, G.M. Zhang and H.L. Zhong, *Curr. Anal. Chem.*, **135**, 9 (2013).
10. Z.G. Chen, G.M. Zhang, X. Chen, J.H. Chen, S.H. Qian and Q. Li, *Analyst*, **722**, 137 (2012).
11. H.S. Zhang and H. Wang, Molecule Probe and Detection Reagents, Science Press, Beijing (2002).