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Determination Of Protein In Human Serum By Acid Brown SR Resonance Light Scattering

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ABSTRACT

A quantitative method was developed for the determination of proteins in aqueous solution. Under optimal conditions, the linear ranges of calibration curves for the determination of bovine serum albumin (BSA), human serum albumin (HSA) and egg albumin (Alb) were 0-1.13 μg ml^{-1}, 0-1.17μg ml^{-1} and 0-1.13μg ml^{-1}, respectively. The detection limits were 15.74 ng ml^{-1} for BSA, 23.34 ng ml^{-1} for HSA and 20.75 ng ml^{-1} for Alb. The method was applied to analysis of total protein in human serum samples collected from the hospital and the results were in good agreement with those reported by a local hospital.

KEYWORDS

Resonance light scattering;
Bovine serum albumin;
Human serum albumin;
Egg albumin;
Acid brown SR.

INTRODUCTION

The quantitative determination of protein is considerably essential in biochemistry and clinical medicine. Among many methods for determination of proteins in samples, Coomassie Brilliant Blue (CBB) [1], Lowry[2] and Bromocresol Green (BCG)[3] are currently widely used. However, they all have some limitations, especially in sensitivity. In recent years, a number of new assays have been reported, such as those based on spectrophotometric[4-7], fluorimetric[8-11], chemiluminescence[12] and electrochemical[13]. In all these of new methods, resonance light scattering (RLS) has been paid much attention because it is characterized by high sensitivity, convenience in performance and simplicity in apparatus (common spectrofluorimeter). When analytical conditions such as wavelength, pH and dye concentration are fixed, the RLS intensity is proportional only to the concentration of scattering particles. This is the quantitative basis for RLS method[14].

Many kinds of dyes have been used for protein

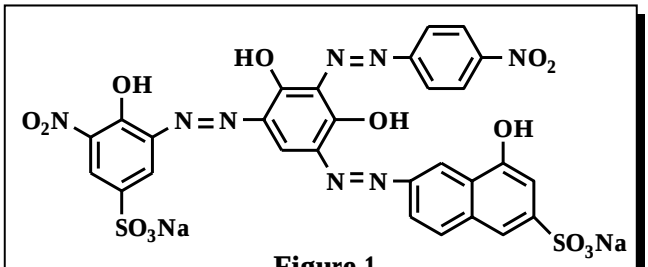

Figure 1

detection by RLS technique, such as arsenazo-DBN [15], bromophenol blue [16], pyrocatechol violet [17], m-acetylchlorophosphonazo[18], amarnath[19], etc. In this report, a tri-azo dye was firstly used as a spectroscopic probe for assay of proteins by RLS technique and a novel method for determination of proteins is presented here. It is based on the binding reaction of proteins with tri-azo probe, ASR (its structure shown in figure1), which was always used in leather industry and has never been used as spectroscopic probe. The weak RLS of ASR can be enhanced greatly by the addition of proteins and the enhanced RLS is proportional to the concentration of proteins. The application of ASR as a probe determination of proteins leads to a sensitive, simple, rapid and selective system.

EXPERIMENTAL

Apparatus

The spectrum and the intensity of RLS were obtained with RF-540 spectrofluorimeter (Shimadzu, Japan) with a 150 w xenon lamp and a 1 cm quartz cell. The excitation and emission bandwidths were 10 nm. The absorbency was obtained with UV-265 spectrophotometer (Shimadzu, Japan). pH measurements were made with a pHs-3C meter (Shanghai, China).

Chemicals

Acid brown SR (ASR) was obtained from Hebei Baoheng Chemistry Company and was directly dissolved in water to prepare a stock standard solution of 6×10^{-4} mol l^{-1}, which was diluted to 3×10^{-5} mol l^{-1} with water to give a working standard solution just prior to use. Triton X-100, gumwater, sodium dodecyl benzene sulfate (DBS), cetyltrimethylammonium bromide (CTAB) were used. Britton-Robinson (B-R) buffer was used to control the acidity of the tested solutions.

BSA(Beijing Aoboxing Biology Company, China), HSA(Huamei Biology Engineering Company, China), Alb(Tianjing Modern Technology and Science Academe, Zhongsan Laboratory, China) were directly dissolved in water to prepare stock standard solutions of 0.1 g l^{-1}, which were stored at 0-4 ^{0}C. Working standard solutions were obtained by diluting the stock standard solutions 100-fold with water just prior to use. The precise concentrations were determined spectrophotometrically at 280 nm with the following $A^{1\%}$ values (the absorbance of a 1% w/v solution with a 1 cm cell, $cm^{-1}mL^{-1}g$): BSA: 6.6[20-21], HSA:5.3[20,22], Alb:7.5[21].

All other chemicals were of analytical-reagent grade. Human serum samples were obtained from the First Center Hospital of Baoding. The serum samples were diluted 100-fold with doubly de-ionized water to prepare stock standard solutions, which were only used in the day when it were prepared. Working standard solutions were obtained by diluting the stock standard solutions 100-fold with water just prior to use.

Standard procedures

B-R buffer (1.5 ml), an appropriate volume of the working standard solutions of ASR and protein (or sample) were mixed, then diluted to 10.0 ml with water and stirred thoroughly. The RLS spectra were obtained with the excitation and emission monochromators of the spectrofluorimeter scanned synchronously (0.0 nm interval between excitation and emission wavelength) through the wavelength range 200-700 nm. All measurements were obtained against a blank treated in the same way without proteins. Based on these spectra, the intensity of RLS was measured with excitation and emission wavelengths at 470 nm.

RESULTS AND DISSCUSSION

Reaction and spectral characteristics

The reaction between ASR and proteins at room temperature occurs rapidly, within 5 min, and the scattering intensity is stable for at least 2 h. The RLS

spectra of ASR and ASR-BSA under optimum conditions are showed in figure 2 (available as SUPPLEMENTARY INFORMATION). For the RLS spectrum of ASR, it has a sharp peak at 470 nm and a lower peak at 405 nm. When BSA coexists in the system, no new peak could be found, while the RLS intensity of ASR was greatly enhanced, especially that of the peak at 470 nm. Such an enhancement effect of protein on RLS intensity of ASR can be interpreted as follows: In acidic aqueous solution (pH=2.48), ASR is presented as negative charge, so that it would be easily bound to positively charged BSA, which leads to the aggregation of ASR on the surface of protein molecule. The aggregation will produce relatively large particles, and result in the enhancement effect of protein on RLS intensity of ASR.

Effect of solution acidity

Figure 3 (available as SUPPLEMENTARY INFORMATION) is the dependence of the RLS intensities for ASR and ASR-BSA on the pH values of the medium. With increasing pH value of the medium, the RLS intensity of ASR has a very slight change, while mixtures of ASR with BSA display different features. When the pH value of the medium is below 3.78, the RLS intensity of ASR-BSA complex has no obvious change, however, if the pH value of the medium is above 3.78, the RLS intensity of ASR-BSA complex decreases sharply. The highest intensity of the ASR-BSA complex is obtained at pH 2.28-3.72, so the optimum acidity (pH=2.48) for the assay was chosen in this range. Furthermore, the effect of buffer amount on the RLS intensity of the system was studied. When the amount of B-R buffer range in 1.0-3.0 ml, the intensity of the ASR-BSA complex reaches a maximum, so 1.5 ml was selected as the optimum amount of buffer.

Effect of ASR concentration

The effects of the ASR concentration on the RLS intensity are shown in figure 4 (available as SUPPLEMENTARY INFORMATION). When the concentration of ASR range in 0.5×10^{-6} mol l^{-1} ~ 4.0×10^{-6} mol l^{-1}, the intensity of ASR remains constant. However, the intensity of ASR-BSA complex displays different features in this range. When the concentration of ASR is below1.50×10^{-6} mol l^{-1}, the intensity of ASR-BSA complex is low which may imply saturation of ASR binding to protein. When the concentration of ASR is $>3.75\times10^{-6}$ mol l^{-1}, the intensity of ASR-BSA complex decreases. This is duo to the higher concentration of the free dye resulting in a higher absorbance and thus less enhancement of the scattering. When the concentration of ASR is in the range 1.50×10^{-6} mol l^{-1} to 3.75×10^{-6} mol l^{-1}, the response of ASR-BSA complex reaches the peak value, and the reading at this condition is constant. Thus, a ASR concentration of 2.40×10^{-6} mol l^{-1} was chosen.

Effect of ionic strength

In order to investigate the ionic strength of medium on the system, different amounts of sodium chloride were added to the system. The results show that the ionic strength of the medium has little effect on the interaction of ASR with BSA when the concentration of sodium chloride is below 3.00×10^{-3} mol l^{-1}. However, when the concentration of sodium chloride is higher than 3.00×10^{-3} mol l^{-1}, the enhanced RLS intensities increased.

Effect of surfactants

The effects of surfactants on RLS are shown in figure 5 (available as SUPPLEMENTARY INFORMATION) (Gumwater), figure 6 (available as SUPPLEMENTARY INFORMATION) (Triton X-100), figure 7 (available as SUPPLEMENTARY INFORMATION) (DBS) and figure 8 (available as SUPPLEMENTARY INFORMATION) (CTAB). Both RLS intensities of ASR and ASR-BSA were enhanced by addition of gumwater, and the enhancements have a linear feature. Addition of Triton X-100 has no obvious effect on the intensity of ASR; however, it has obvious effect on the RLS intensity of ASR-BSA complex. When the concentration of Triton X-100 < 0.01%, the RLS intensity increased sharply; and when the concentration of Triton X-100 > 0.01%, the RLS intensity remained almost. constant However, addition of DBS and CTAB results in a very complex change on the RLS intensity. The reason for these phenomena is not clear yet and

worthy of further study. In this work, no surfactants were added in order to simplify the proposed method

Interfering substance

The influence of possible substances in the samples, such as amino acids, urea, glucose and common metal ions, was studied. As shown in TABLE 1 (available as SUPPLEMENTARY INFORMATION), it seems to interfere with this assay for some of the ions and amino acids tested. However, due to the real serum samples would be diluted 105-fold before RLS experiment, the concentration of these interfering substances would be very low so that they could not interfere the determination, so no special separation procedure was needed before sample determination. And it indicates that this assay is simple and selective.

Calibration equations

The regression equations made by BSA, HSA and Alb are presented in TABLE 2 (available as SUPPLEMENTARY INFORMATION).

Measurement of samples

The determination of protein is very important in clinical analysis and biochemistry. By using the present method and choosing HSA as the standard, proteins in samples of human serum were measured. The results are shown in TABLE 3 (available as SUPPLEMENTARY INFORMATION). Compared with the data obtained from the hospital, the results are very satisfactory.

CONCLUSION

ASR is a tri-azo dye and it has never been used as a spectroscopic probe. It is demonstrated here that this reagent performs well in the determination of proteins. Based on the fact that the weak RLS intensity of ASR can be enhanced greatly by the addition of proteins, the new method for determination of proteins was developed. The determination results of this method are very close to the data obtained from hospital, which confirmed that this novel RLS approach could undoubtedly be used in the determination of proteins. Compared with other general methods, the main advantages of this method are sensitivity, simplicity, rapidity and cheapness.

SUPPLEMENTARY INFORMATION AVAILABLE STATEMENT

Following information is available as SUPPLEMENTARY INFORMATION.

- TABLE 1: Effects of interfering substances on RLS
- TABLE 2 : Analytical parameters for the determination of protein by RLS
- TABLE 3: Determination results for human serum samples
- Figure 2: RLS spectra for ASR and ASR-BSA under optimum conditions: $C_{ASR}=2.40\times10^{-6}$ mol l^{-1} $C_{BSA}=0.94$ μg.ml^{-1}; pH=2.48; $V_{B-R}=1.5$ ml
- Figure 3: Effect of pH on RLS intensity of ASR and ASR-BSA: 1: ASR; 2: ASR-BSA; 3: Difference between ASR and ASR-BSA; $C_{ASR}=3.00\times10^{-6}$ mol l^{-1}; $C_{BSA}=0.94$ μg ml^{-1}; $V_{B-R}=2$ ml
- Figure 4: Effect of ASR concentration on RLS intensity of ASR and ASR-BSA: 1: ASR; 2: ASR-BSA; 3: Difference between ASR and ASR-BSA; $C_{BSA}=0.94$ μg ml^{-1}; pH=2.48; $V_{B-R}=1.5$ ml
- Figure 5: Effect of Gumwater on RLS intensity: $C_{ASR}=2.40\times10^{-6}$ mol l^{-1}; $C_{BSA}=0.94$ mg ml^{-1}; pH=2.48; $V_{B-R}=1.5$ ml
- Figure 6: Effect of Triton X-100 on RLS intensity: $C_{ASR}=2.40\times10^{-6}$ mol l^{-1}; $C_{BSA}=0.94$ μg ml^{-1}; pH=2.48; $V_{B-R}=1.5$ ml
- Figure 7: Effect of DBS on RLS intensity: $C_{ASR}=2.40\times10^{-6}$ mol l^{-1}; $C_{BSA}=0.94$ μg ml^{-1}; pH=2.48; $V_{B-R}=1.5$ ml
- Figure 8: Effect of CTAB on RLS intensity: $C_{ASR}=2.40\times10^{-6}$ mol l^{-1}; $C_{BSA}=0.94$ μg ml^{-1}; pH=2.48; $V_{B-R}=1.5$ ml

REFERENCES

[1] M.M.Bradford; Anal.Biochem., **72**, 248 **(1976)**.
[2] O.H.Lowery, N.H.Rosebrough, A.L.Farr; J.Biol. Chem., **193**, 265 **(1951)**.
[3] P.H.Lolekha, W.Charoenpol; Clin.Chem., **20**, 617 **(1974)**.
[4] H.S.Soedjak; Anal.Biochem., **220**, 142 **(1994)**.
[5] A.A.Waheed, P.D.Gupta; Anal.Biochem., **223**, 249 **(1996)**.
[6] K.Zhu, K.A.Li, S.Y.Tong; Anal.Lett., **29**, 575 **(1996)**.
[7] Z.X.Guo, Y.M.Hao, X.Cong, H.X.Shen; Anal. Chim.Acta, **403**, 225 **(2000)**.
[8] J.Nakamura, S.Lgarashi; Anal.Lett., **29**, 981 **(1996)**.
[9] N.Li, K.A.Li, S.Y.Tong; Anal.Biochem., **233**, 151 **(1996)**.
[10] M.A.Kessler, A.Meinitzer, O.S.Wolfbeis; Anal. Biochem., **248**, 180 **(1997)**.
[11] D.H.Li, H.H.Yang, H.Zhen, Y.Fang, Q.Z.Zhu, J.G. Xu; Anal.Chim.Acta, **401**, 185 **(1999)**.
[12] K.Tsukagoshi, Y.Okumura, H.Akasaka, R.Nakajima, T.Hara; Anal.Sci., **525**, 279 **(1996)**.
[13] H.M.Zhang, Z.W.Zhu, N.Q.Li; Frenius' J.Anal.

Full Paper

Chem., **363**, 408 **(1999)**.
[14] L.J.Dong, R.P.Jia, Q.F.Li, X.G.Chen, Z.D.Hu; Analyst, **126**, 707 **(2001)**.
[15] Q.F.Li, X.G.Chen, H.Y.Zhang, C.X.Xue, S.H.Liu, Z.D.Hu; Analyst, **25**, 1483 **(2000)**.
[16] C.Q.Ma, K.An.Li, S.Y.Tong; Anal.Biochem., **239**, 86 **(1996)**.
[17] X.Cong, Z.X.Guo, X.X.Wang, H.X.Shen; Anal.Chim. Acta, **444**, 205 **(2001)**.
[18] Q.F.Li, S.H.Liu, H.Y.Zhang, X.G.Chen, Z.D.Hu; Anal. Lett., **34**, 1133 **(2001)**.
[19] X.X.Wang, H.X.Shen, Y.M.Hao; Chinese J.Anal. Chem., **28**, 1388 **(2000)**.
[20] D.M.Kirschenbaum; 'Handbook of Biochemistry, Selected Data for Molecular Biology', H.Sober , 2nd Ed., Chemical Rubber Co., Cleveland, OH, C-71-C-98 **(1970)**.
[21] G.D.Fasman; 'CRC Practical Handbook of Biochemistry and Molecular Biology Proteins', CRC Press, Boca Raton, FL, **(1976)**.
[22] D.B.Wetlaufer; Adv.Protein Chem., **17**, 378 **(1962)**.