Resonance Rayleigh Scattering Spectra of Thorium(IV)-bisazo Dye of Chromotropic Acid-protein Systems and Their Analytical Applications

FAN, Li(范莉) LIU, Shao-Pu*(刘绍璞) YANG, Da-Cheng(杨大成) LUO, Hong-Qun(罗红群)
Institute of Environmental Chemistry, Southwest China Normal University, Chongqing 400715, China

In acidic medium, thorium(IV) can react with a bisazo dye of chromotropic acids such as arsenazo III (AA III), arsenazo M (AA M), chlorophosphonazo III (CPA III) and chlorosulphonphenol S (CSP S) to form an anionic chelate which further interacts with some proteins to produce a complex. This results in a significant enhancement of intensity of the resonance Rayleigh scattering (RRS) and the appearance of a new RRS spectrum. There are a few obvious RRS peaks in the range of 400—470 nm and the most intensive peak of them is located at 470 nm. The intensity of RRS is directly proportional to the concentration of protein in the range of 0—1.6 $\mu g \cdot mL^{-1}$ for Th(IV)-CPA III system, 0—2.8 $\mu g \cdot mL^{-1}$ for Th(IV)-AA M system, 0—2.0 $\mu g \cdot mL^{-1}$ for Th(IV)-AA III system and 0—0.28 $\mu g \cdot mL^{-1}$ for Th(IV)-CSP S system, respectively. The detection limits for BSA (3σ) are 10.7 $ng \cdot mL^{-1}$ for Th(IV)-CPA III, 6.3 $ng \cdot mL^{-1}$ for Th(IV)-CSP S, 13.6 $ng \cdot mL^{-1}$ for Th(IV)-AA III and 22.1 $ng \cdot mL^{-1}$ for Th(IV)-AA M, respectively. This new RRS method has high sensitivity and fairly good selectivity and can be applied to the direct determination of proteins in human serum with satisfactory results.

Keywords resonance Rayleigh scattering, protein, arsenazo III, arsenazo M, chlorophosphonazo III, chlorosulphonphenol S, Th(IV)

Introduction

The determination of proteins is important in biochemical study, clinical treatment and food analysis. As it is known, the common methods used to determine the protein quantitatively at present are spectrophotometry and fluorospectrophotometry.[1] Recently, resonance Rayleigh scattering (RRS), a new analytical technique, has been applied to the determination of some biological macromolecules such as nucleic acids and proteins,[2-8] mainly based on the aggregation of dye chromophore on the biological macromolecules which can further give rise to a strong RRS.[3] The most common used dyes are acidic triphenylmethane dyes and acidic xanthene dyes such as chrome aurol S (CAS),[9] bromophenol blue (BPB),[10] pyrogallol red (PR)[11] and bromopyrogallol red (BPR).[12] In addition, arsenazo III (AA III), a bisazo dye, has also been utilized to the determination of proteins with RRS method.[13] Recently, we found that besides AA III, some other bisazo dyes of chromotropic acid such as arsenazo M (AA M) with arsono group, chlorophosphonazo III (CPA III) with phosphono group and chlorosulphonphenol S (CSP S) with *O*,*O'*-dihydroxy group on the aromatic ring *etc.* could also be used to the determination of proteins by RRS method. Although the intensity of RRS of above-mentioned dyes is very faint in weak acidic medium, much more strong intensity of RRS has been observed when they reacted with some proteins to form complex. When Th(IV) was added into the same solution, the intensity of RRS could be enhanced greatly due to its interaction with these dyes and some proteins which further form a ternary complex of dye-Th(IV)-protein. As the result, the sensitivity was increased for 1—2 times than that of the corresponding binary complex of dye-protein. The intensity of RRS is directly proportional to the concentration of protein in the range of 0—1.6 $\mu g \cdot mL^{-1}$ for Th(IV)-CPA III system and between 0—0.28 and 0—2.8 $\mu g \cdot mL^{-1}$ corresponding to other three Th(IV)-dye systems.

In this work, the RRS spectral characteristics of the combination products of above four Th(IV)-bisazo dyes of chromotropic acid with proteins, the optimum condition of the reactions and the primary influencing factors have been investigated comprehensively. The new RRS method with high sensitivity and fairly good selectivity can be applied to the direct determination of proteins in human serum.

Experimental

Apparatus and reagents

A Shimadzu RF-540 spectrofluorophotometer (Kyoto, Japan) was used. The parameters were: slit (EX/EM): 10.0 nm/10.0 nm; low sensitivity.

Standard solutions of proteins: 20 $\mu g \cdot mL^{-1}$ bovine serum albumin (BSA) and 30 $\mu g \cdot mL^{-1}$ cellulase (trichoderma viride G) (Cel) (Shanghai Lizhu Dongfeng Biotechnology Limited Company); 30 $\mu g \cdot mL^{-1}$ human serum albumin (HSA, Shanghai Biological Product Research Institute); 50

* E-mail: liusp@swnu.edu.cn
Received April 3, 2002; revised July 18, 2002; accepted September 16, 2002.
Project supported by the National Natural Science Foundation of China (No. 29875019).

$\mu g \cdot mL^{-1}$ α-chymotrypsin (α-Chy, IGM Company); 30 $\mu g \cdot mL^{-1}$ ovalbumin (Ova, Sigma Company); 20 $\mu g \cdot mL^{-1}$ lysozyme (Lys), 30 $\mu g \cdot mL^{-1}$ pepsin (Pep) and 40 $\mu g \cdot mL^{-1}$ trypsin (Try) (Huamei Biological Engineering Company); 40 $\mu g \cdot mL^{-1}$ α-amylase (*bacellus subtilis*) (α-Amy) and 20 $\mu g \cdot mL^{-1}$ haematin hydrochloride (Hae) (Shanghai Dongfeng Biochemistry Technical Company) were prepared in water and stored at 1—4 ℃.

Dye solutions: arsenazo III (AA III, Chemical Plant of Beijing Normal University), arsenazo M (AA M, Beijing Chemical Plant), chlorophosphonazo III (CPA III, Chemical Plant of Shanghai Normal University), chlorosulphonphenol S (CSP S, Chemical Plant of East China Normal University) and Coomassie brilliant blue G-250 (CBB G-250, Shanghai Chemistry Reagent Company of China Pharmaceutical Corp) were directly dissolved in water to prepare stock solution of 1×10^{-3} mol·L^{-1} each, and the working solutions of 2×10^{-5} mol·L^{-1} or 1×10^{-4} mol·L^{-1} were obtained by diluting the stock solution with water.

Thorium nitrate solution was directly dissolved in water to prepare stock solution of 1.0×10^{-2} mol·L^{-1} Th(IV), and the working solution of 1.0×10^{-4} mol·L^{-1} was obtained by diluting the stock solution with water.

0.2% (*w/V*) of triton X-100, polyvinyl alcohol, gum acacia and emulsify OP solubilizing solutions, 0.1 mol·L^{-1} sodium citrate-hydrochloride buffer solution (pH 1.0—4.4) and 0.1 mol·L^{-1} sodium acetate-hydrochloride buffer solution (pH 0.6—5.2) were used.

All the reagents used are of analytical grade and water is doubly distilled.

General procedure

To a 10 mL of dry calibrated flask, appropriate amount of dye, suitable amounts of Th(IV), buffer solution, triton X-100 solution and protein were added in proper order as shown in Table 1 and diluted to the mark with water. After mixing and setting the solution aside for 10 min, the RRS spectra were recorded with synchronous scanning at $\lambda_{ex} = \lambda_{em}$ (*i.e.*, $\Delta\lambda = 0$ nm), and the RRS intensity I for the reaction product and I_0 for the reagent blank at each λ_{detn}^{RRS} (chosen as the determination wave length) were measured, $\Delta I = I - I_0$.

Results and discussion

RRS *spectral characteristics*

The experimental results show that the above four systems have similar RRS spectral characteristics (Table 2). For an illustration, Figs. 1 and 2 show the RRS spectra of Th(IV)-CPA III-BSA system. From Table 2, Figs. 1 and 2, it can be concluded that: (1) the intensities of RRS of protein, dye and Th(IV)-dye chelate solution are all very faint; (2) under the measurement conditions, the intensity of RRS is enhanced to a certain degree because the proteins combine with the dyes to form a binary complex; (3) if there are suitable amounts of Th(IV), the RRS will be significantly strengthened because of the formation of ternary complex of Th(IV)-dye-protein; (4) no matter how different the analytical functional-group of dye is, the RRS spectral characteristics are similar, in which all have strong RRS in the range of 400—470 nm and the maximum RRS peaks are all at 470 nm. 400 or 415 nm is chosen as the determination wavelength rather than 470 nm because the RRS peak at 400 or 415 nm is smoother than that at 470 nm, although the RRS intensity at 400 or 415 nm is slightly lower.

Optimum conditions of the reactions

Effect of solution acidity The solution acidity should be adjusted to favor the formation of Th(IV)-dye anionic chelate and the combination of anionic chelate with proteins. The experimental results are shown in Fig. 3. It can be seen that all reactions can proceed in acid medium at pH 1—3, which is ahead of the isoelectric point of BSA (p*I* 4.7). For

Table 1 Experimental conditions

System	pH	Th(IV) added (mL)	Dye added (mL)	Triton X-100 added (mL)	Buffer solution added (mL)
CPA III-Th(IV)-BSA	1.9	1.0	1.0	1.0	1.0
CSP S-Th(IV)-BSA	2.3[a]	3.0	1.0	0.6	1.2
AA III-Th(IV)-BSA	1.4	0.2	1.0	1.0	1.5
AA M-Th(IV)-BSA	1.4	0.2	1.0	1.0	1.0

[a] 0.1 mol·L^{-1} sodium acetate-hydrochloride buffer solution was used.

Table 2 Spectral characteristics of RRS (1.0 $\mu g \cdot mL^{-1}$ BSA)

System	λ_{max} (nm)	$\lambda_{det\,n}^{RRS}$ (nm)	I/I_0	Other peaks of RRS (nm)
AA III-Th(IV)-BSA	470	415	23.2/3.6	290, 400, 415, 450, 483, 492, 572
AA M-Th(IV)-BSA	470	400	16.3/4.9	290, 400, 415, 450, 483, 492, 530, 572
CPA III-Th(IV)-BSA	470	415	35.7/2.8	290, 400, 415, 450, 483, 492, 510
CSP S-Th(IV)-BSA	470	400	24.6/13.8[a]	290, 400, 415, 450, 483, 492

[a] 0.2 $\mu g \cdot mL^{-1}$ BSA.

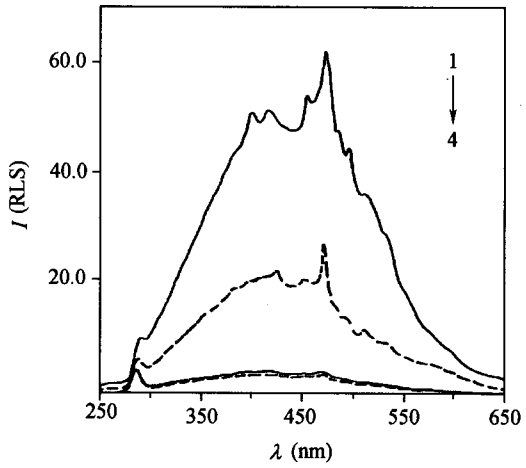

Fig. 1 Comparison of RRS spectra of binary system with ternary complex system. (1) CPA III-Th (IV)-BSA system; (2) CPA III-BSA system; (3) CPA III-Th(IV) system; (4) CPA III system. BSA: 1.6 $\mu g \cdot mL^{-1}$.

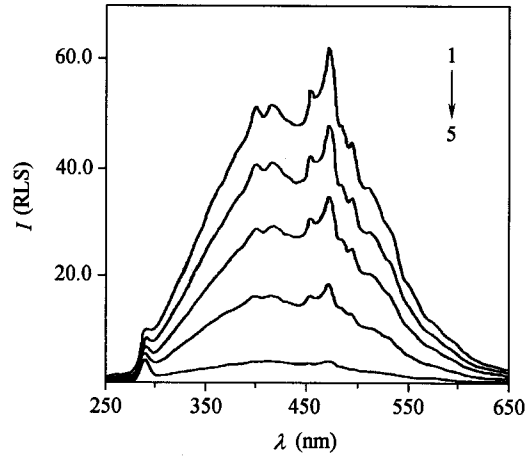

Fig. 2 RRS spectra of CPA III-Th(IV)-BSA system (pH 1.9). BSA ($\mu g \cdot mL^{-1}$): (1) 1.6; (2) 1.2; (3) 0.8; (4) 0.4; (5) 0.0.

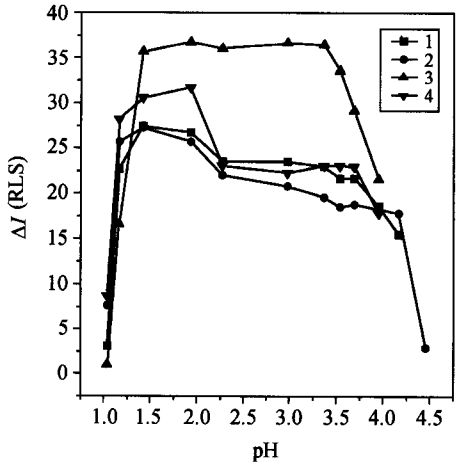

Fig. 3 Effect of solution acidity. Triton X-100: 0.02%, Th(IV): 2×10^{-4} $mol \cdot L^{-1}$, Dye: 2×10^{-6} $mol \cdot L^{-1}$, BSA: 2.0 $\mu g \cdot mL^{-1}$. (1) AA III system; (2) AA M system; (3) CPA III system; (4) CSP S system.

this reason there are positive charges at its nitrogen atoms of basic amino acid on the peptide chains, and therefore, BSA is a large cation with many positive charges. In this case, the two SO_3H groups on the aromatic ring of chromotropic acid of the dye completely dissociated and some other acidic groups such as PO_3H, AsO_3H and OH bearing on the benzene ring also probably dissociated to some extent, the dyes all exist in the anion species with 2 or 3 charges. Meanwhile, AA III and CPA III *etc*. can form complexes with Th(IV) in the ratio of $1:2$[14] and exist as greater anions which can combine with proteins by virtue of electrostatic forces and hydrophobic interaction, then cause the significant enhancement of RRS. If the pH value goes lower, the dissociation of dye will be restrained. But when the pH value is much higher, the positive charge of protein will reduce and the complex is not apt to be formed. Therefore it is unfavorable to the combination reaction when pH is too high or too low. But the range of the pH value is distinct for different dye system. Among them, the pH range of the CPA III system is the broadest (pH 1.4—3.4), that of the CSP S system is the second (pH 1.8—2.6), and those of the AA M system and the AA III system are narrow (pH 1.2—1.9 and pH 1.4—1.9).

Effect of solubilizing agents In order to improve the sensitivity of the above-mentioned systems, the effect of some solubilizing agents such as triton X-100, polyvinyl alcohol, gum acacia and emulsified OP on the intensity of RRS was tested. Triton X-100, PVA and emulsified OP can reinforce the sensitivity of the reaction, but the RRS intensity for the reagent blank of PVA is too high, and gum acacia decreases the RRS intensity. So 0.2% triton X-100 solution was chosen as solubilizing agent. When its volume added is 0.6—1.0 mL, the ΔI reaches a maximum value and keeps constant.

Effect of Th(IV) *concentration* As shown in Fig. 4, with the increase of Th(IV) concentration, the RRS intensity enhances gradually and keeps stable in certain concen-

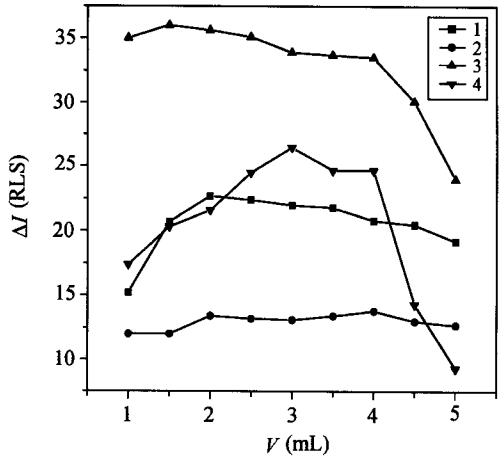

Fig. 4 Effect of Th(IV) concentration. Dye: 2×10^{-6} $mol \cdot L^{-1}$, BSA: 2.0 $\mu g \cdot mL^{-1}$, Th(IV): 2×10^{-4} $mol \cdot L^{-1}$ (AA III and AA M), 2×10^{-5} $mol \cdot L^{-1}$ (CPA III and CSP S). (1) AA III system; (2) AA M system; (3) CPA III system; (4) CSP S system.

tration range, but if Th(IV) concentration is too high, on the other hand, the RRS intensity decreases. The reason is that if the concentration of Th(IV) is too low, its reaction with dyes will not perform completely and the scattering intensity gets lower. If the concentration is too high, the complex of Th (IV)-dye in the ratio of 1:2 is not easy to be formed, thus the RRS intensity decreases due to the size of particles too large to produce Rayleigh scattering.[2]

Effect of ion strength The effect of the ion strength on the RRS intensity was also investigated. It was demonstrated that ΔI value will reduce a little with the add of NaCl. It hints that the competitive action of the small ions, such as Na^+ with positive charge and Cl^- with negative charge for the reaction between the chelate anion and protein, is feeble. So it is unnecessary to add other salts such as NaCl into the system to adjust the ion strength.

Reaction time and stability

Although the reactions accomplish rapidly at room temperature, the RRS intensity can remain constant for at least 3 h. It is believed that the influence of temperature is not obvious at 10—35 ℃ and the reaction condition is not very exacting.

Sensitivities of the methods

Under the optimum condition, the reactions between different dye-Th(IV) chelate anions and proteins such as BSA, HSA, α-Chy, Ova, Lys, Pep, Try, Cel, α-Amy and Hae were examined carefully (Table 3). Pep, Try and Cel can not react with the chelate anions. Although the RRS intensity enhances obviously with the addition of Hae, but linear relationship between ΔI_{RRS} and Hae can not be obtained. All the other proteins can exhibit fairly good sensitivity. The detection limits are in the range of 6.30—22.1 $ng \cdot mL^{-1}$, which are improved greatly compared with that of the binary system under the same conditions.[15]

The reason why the intensity of RRS is potentiated rapidly is that when dye molecules change from H_6L^{2-} or H_5L^{3-} to $[Th(H_5L)_2]^{2-}$, the volume of the complex molecules augments doubly and the molecular weight rises significantly. The reaction equations are as follows:

$$2H_5L^{3-} + Th^{4+} \rightleftharpoons [Th(H_5L)_2]^{2-} \quad (1)$$

$$2H_6L^{2-} + Th^{4+} \rightleftharpoons [Th(H_5L)_2]^{2-} + 2H^+ \quad (2)$$

In addition, the molar absorptivity of the molecules increases enormously after the formation of chelate. According to the formula,[16]

$$I_{RRS} = I_0 \frac{(2.303)^2 \cdot 1000cn}{N_A} \left\{ \frac{\varepsilon^2(\lambda_0)}{4\lambda_0^2} + \left[\frac{1}{\pi} \int_0^\infty \frac{\varepsilon(\lambda)}{\lambda_0^2 - \lambda^2} d\lambda \right]^2 \right\} c_v \quad (3)$$

Where $\varepsilon(\lambda)$ is the molar absorptivity. And the simplified Rayleigh formula is[17]

$$I_{RRS} = KcMI_0 \quad (4)$$

Where M is the molecular weight.

From Eqs. (3) and (4), It can be seen that the growth of the molecular weight and the augment of molar absorptivity are advantageous to the enhancement of RRS. It is believed that the intensity of RRS of ternary system is stronger than that of homological binary system. Among the four systems, the CPA III system has the highest sensitivity and the widest linear range, its detection limits ($\sigma = 3$) for different protein are 2.9—16.5 $ng \cdot mL^{-1}$. Lys is the most sensitive, Amy is the lowest, but its linear range is wider. For the dye-Th(IV)-BSA systems, the CPA III-Th(IV)-BSA system is the best considering the ranges of pH value, the linear ranges and the correlation coefficients, so it was chosen to investigate the effect of coexisting substances and the analytical application.

Table 3 Linear regression equations of RRS and the detection limits for the proteins

System	Linear regression equation (c: $\mu g \cdot mL^{-1}$)	Correlation coefficient (r)	Linear range ($\mu g \cdot mL^{-1}$)	Detection limit (3σ: $ng \cdot mL^{-1}$)
AA III-Th(IV)-BSA	$\Delta I = 28.7c + 3.56$	0.9975	0—0.40	13.6
	$\Delta I = 15.7c + 7.45$	0.9997	0.40—2.00	
AA M-Th(IV)-BSA	$\Delta I = 11.4c + 4.88$	0.9988	0—2.80	22.1
CSP S-Th(IV)-BSA	$\Delta I = 54.4c + 13.80$	0.9999	0—0.28	6.3
CPA III-Th(IV)-BSA	$\Delta I = 23.4c + 2.73$	0.9973	0—0.40	10.7
	$\Delta I = 33.8c + 1.90$	0.9999	0.40—1.60	
CPA III-Th(IV)-HSA	$\Delta I = 11.0c - 0.82$	0.9987	0—2.70	15.0
CPA III-Th(IV)-Ova	$\Delta I = 10.5c - 0.087$	0.9998	0—3.12	15.7
CPA III-Th(IV)-Lys	$\Delta I = 57.2c + 0.44$	0.9963	0—0.56	2.9
CPA III-Th(IV)-Chy	$\Delta I = 12.0c + 0.40$	0.9997	0—2.64	13.7
CPA III-Th(IV)-Amy	$\Delta I = 10.0c - 0.35$	0.9999	0—3.04	16.5

Effect of different metal ions

In pH 1—2 acidic medium, the bisazo dyes of chromotropic acid form chelate anions not only with Th(IV) but also with some other metal ions which further react with protein to give rise to intensive RRS. After checking eleven metal ions, it is found that Bi(III), U(VI) and Mo(VI) result in significant enhancement of RRS, but others do not (Table 4). Therefore, Bi(III), U(VI) and Mo(VI) can also be utilized to determine protein in such ternary system.

Selectivity and analytical application of the method

Selectivity of the method Under appropriate conditions, the influence of coexisting substances on the determination of BSA was investigated and the results are shown in Table 5. It can be seen that common metal ions and acid group ions along with tens fold of amino acid have no obvious interference on the determination. Therefore, the method has fairly good selectivity.

Determination of total protein in human serum Human serum samples were obtained from three different donors in the hospital of Southwest China Normal University. These samples were diluted 5000-fold with doubly distilled water as total protein samples. To a 10 mL of dry calibrated flask, 1.0 mL of CPA III solution, 1.0 mL of triton X-100 solution, 1.0 mL of Th(IV) solution and 1.0 mL of buffer solution were added sequentially. After mixing and setting the solution aside for 5 min, 0.8 mL of human serum total protein sample previously prepared was added and diluted to the mark with water. After mixing and setting the solution aside for another 2 min, the RRS intensity of the sample was recorded as mentioned above. The results are shown in Table 6.

In addition, we also investigated the recovery of protein of sample 1 with the standard addition method. The experimental results show that the average recovery for six measurements is 99.4% and the relative standard deviation (RSD) is 1.02%. By comparing the results with the generally performed Bradford method using CBB G-250, it was found that the CPA III-Th(IV) method for the determination of the total content of proteins is reliable, sensitive and practical.

Table 4 Effect of different metal ions

Metal ion	Appropriate concentration ($\mu g \cdot mL^{-1}$)	ΔI	Metal ion	Appropriate concentration ($\mu g \cdot mL^{-1}$)	ΔI
Th(IV)	6	59.1	Ba(II)		221.0
Mo(VI)	3	41.3	Sn(IV)	2	26.3
Al(III)	1	24.5	Ni(II)	1	23.5
Cr(III)	1	21.7	Ga(III)	2	33.5
Bi(III)	10	58.6	Sn(II)	2	23.3
U(VI)	10	59.6			

Table 5 Effect of coexisting substances (BSA: 1.0 $\mu g \cdot mL^{-1}$)[a]

Coexisting substance	Concentration tolerated ($\mu g \cdot mL^{-1}$)	Change of ΔI value (%)	Coexisting substance	Concentration tolerated ($\mu g \cdot mL^{-1}$)	Change of ΔI value (%)	Coexisting substance	Concentration tolerated ($\mu g \cdot mL^{-1}$)	Change of ΔI value (%)
Glycine	70	4.9	SiO_3^{2-}	100	-8.3	Ba(II)	1	5.9
L-Histidine	20	6.5	PO_4^{3-}	8	-6.0	Cu(II)	20	7.6
L-Leucine	50	8.9	$C_2O_4^{2-}$	1	8.5	Ni(II)	4	8.9
L-Tryptophan	30	2.6	Bi(III)	0.5	8.6	Fe(III)	10	7.5
L-Phenylalanine	50	-8.2	Cr(III)	2	7.7	Mg(II)	10	8.3
L-Isoleucine	50	-6.9	Mn(II)	150	8.5	Sn(IV)	2	7.2
DL-Threonine	50	8.9	Sn(II)	1	2.8	Co(II)	30	5.6
DL-Cysteine	30	-2.6	Be(II)	50	4.9	Pb(II)	100	8.9
DL-Aspartic acid	50	6.9	UO_2^{2+}	0.1	8.2	Zn(II)	100	-9.8
D-Threonine	50	-5.6	Sb(III)	200	4.5	Ca(II)	20	-7.9
D-Tryptophan	50	8.9	Mo(VI)	1.5	-3.6	Sr(II)	40	7.3
Se(IV)	50	4.9	W(VI)	10	2.6	Cd(II)	60	6.6
NO_2^-	100	-8.3	V(V)	100	2.1	Al(III)	10	9.5
$HAsO_4^{2-}$	10	4.9						

[a] CPA III: 2×10^{-6} mol·L^{-1}, Th(IV): 1×10^{-5} mol·L^{-1}, Triton X-100: 0.02%, buffer solution: 1.0 mL (pH 1.9), BSA: 1.0 $\mu g \cdot L^{-1}$, λ = 415 nm.

Table 6 Determination of proteins in human serum

Sample	CBB G-250 method		CPA III-Th(IV) method		Relative error (%) (relative to CBB G-250 method)
	RSD (%) ($n=6$)	Protein content in sample ($mg \cdot mL^{-1}$)	RSD (%) ($n=4$)	Protein content in sample ($mg \cdot mL^{-1}$)	
1	2.3	72.0	1.0	69.5	-3.5
2	1.5	71.4	1.5	70.1	-1.8
3	2.0	70.9	1.4	71.0	0.1

References

1 Yang, R.; Liu, S. P. *Chin. J. Anal. Chem.* **2001**, *29*, 232 (in Chinese).

2 Pasternack, P. F.; Collings, P. F.; Giannetto, A.; Gibbs, E. J. *J. Am. Chem. Soc.* **1993**, *115*, 5393.

3 Pasternack, R. F.; Collings P. J. *Science* **1995**, *269*, 935.

4 Li, Y. F.; Huang, C. Z.; Hu, X. L. *Chin. J. Anal. Chem.* **1998**, *26*, 1508 (in Chinese).
Liu, S. P.; Hu, X. L.; Luo, H.Q.; Fan, L. *Sci. China, Ser. B* **2002**, *32*, 18 (in Chinese).

5 Huang, C. Z.; Li, K. A.; Tong, S. Y. *J. Am. Chem. Soc.* **1997**, *69*, 514.

6 Ma, C. Q.; Liu, Y.; Li, K. A.; Tong, S. Y. *Chin. Sci. Bull.* **1999**, *44*, 682 (in Chinese).

7 Liu, S. P.; Liu, Q.; Yang, R. *Anal. Sci.* **2001**, *17*, 239.
Liu, S. P.; Yang, R.; Liu, Q. *Anal. Sci.* **2001**, *17*, 243

8 Zhang, W. J.; Xu, H. P.; Xue, C. X.; Chen, X. G.; Hu, Z. D. *Anal. Lett.* **2001**, *34*, 553
Zhang, W. J.; Xu, H. P.; Wu, S. Q.; Chen, X. G.; Hu, Z. D. *Analyst* **2001**, *126*, 513

9 Wei, Y. J.; Li, K. A.; Tong, S. Y. *Acta Chim. Sinica* **1998**, *56*, 295 (in Chinese).

10 Ma, C. Q.; L,i K. A.; Tong, S. Y. *Anal. Biochem.* **1996**, *239*, 86.

11 Hu, X. L.; Feng, P.; Li, Y. F. *J. Southwest China Normal Univ.* (*Nat. Sci. Ed.*) **1998**, *23*, 561 (in Chinese).

12 Ma, C. Q.; Li, K. A.; Tong, S. Y. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 129.

13 Yao, G.; Li, K. A.; Tong, S. Y. *Anal. Lett.* **1998**, *31*, 1689.

14 Zeng, Y. E.; Zhang, H. S.; Chen, Z. H. *Modern Inorganic Chemistry Reagents Handbook*, Chemistry Industry Press, Beijing, **1989**, 23 (in Chinese).

15 Fan, L.; Liu, S. P.; Long, X. F.; Hu, X. L. *Chin. J. Anal. Chem.* **2002**, *28*, 81 (in Chinese).

16 Anglister, J.; Steinberg, I. Z. *J. Chem. Phys.* **1983**, *78*, 5358.

17 Edited by Chinese Encyclopedia Editorial Board, *Chinese Encyclopedia: Biology II*, Chinese Encyclopedia Press, Beijing, **1991**, p. 1394 (in Chinese).

(E0204031 LI, L. T.; DONG, L. J.)