Resonance Scattering Spectral Determination of HSA with a New Scattering Enhanced Reagent of $K_3[Fe(CN)_6]$

JIANG, Zhi-Liang*,[a,b](蒋治良) **PENG, Zhong-Li**[b](彭忠利) **LIU, Shao-Pu**[a](刘绍璞)

[a] *Institute of Environmental Chemistry, Southwest-China Normal University, Chongqing 400715, China*

[b] *Department of Resource and Environmental Science, Guangxi Normal University, Guilin, Guangxi 541004, China*

A new resonance scattering spectral (RSS) method for the determination of human serum album (HSA) has been proposed with the resonance scattering enhanced reagent of $K_3[Fe(CN)_6]$. In the medium of HCl (2.0×10^{-3} mol/L), HSA may combine with $[Fe(CN)_6]^{3-}$ by intermolecular forces (mainly by electrostatic force) to form $\{[Fe(CN)_6]_n^{3-}\text{-}HSA^{m+}\}_k$ nanoparticle of the ion-association complexes of $HSA^{m+}\text{-}[Fe(CN)_6]_n^{3-}$. There is a strongest resonance scattering intensity at 351 nm, owing to the existence of the resonance scattering of the nanoparticle, $[Fe(CN)_6]^{3-}$ molecular absorption and the non-distribution of the emission intensity of Xe lamp in the range of 200—1000 nm. In addition, two resonance scattering peaks at 470 and 700 nm were observed. The HSA concentration in the range of 0—12 μg/mL is linear to the resonance scattering intensity at 351 nm. The determination limit of this method is 0.1 μg/mL HSA, about ten-fold lower than that of Coomassie brilliant blue protein assay. This method has been used for the determination of HSA in human serum and synthetic samples with satisfactory results. The mechanism of enhanced resonance scattering light, the TEM of the particle, the concepts of quasi-elastic absorption and un-elastic absorption were also discussed.

Keywords nanoparticle, ion-association complex, resonance scattering spectroscopy, HSA, $K_3[Fe(CN)_6]$

Protein determination is very important to biochemistry and bioanalytical chemistry, and an analytical item of quality controls in the separation or purification of biological and chemical pharmaceuticals and that of food examination. Compared with common spectrophotometric method such as biuret and lowry assay[1] and spectrophotometric method based on the combination of proteins with dye sush as CBB assay,[2] resonance scattering spectral (RSS) method (also called resonance light scattering[3]) developed recently was proved to be a sensitively and selectively quantitative determination for proteins,[4-9] which was based on the stacking of organic dye on proteins. However, the use of metal complex anions is not more. It is rare reports to use transmission electron microscope (TEM) in the study of the protein's nanoparticles in RSS analysis of proteins. Here, by using resonance scattering technique and TEM, a new protein assay was established which was first based on the combination of $[Fe(CN)_6]^{3-}$ with protein to supramolecule or particle in large size. It would improve the development of resonance scattering technique and offer a new approach for protein assay. The concepts of inelastic absorption and quasi-elastic absorption have been proposed in this work. The cause of producing the resonance scattering peak has also been considered.

Experimental

Apparatus

The resonance scattering spectrum and the intensity of resonance scattering were measured with a Shimadzu RF-540 fluorescence spectrophotometer (Kyoto, Japan).

* E-mail: zljiang@mailbox.gxnu.edu.cn

Received December 5, 2001; revised June 14, 2002; accepted July 24, 2002.

Project supported by the National Natural Science Foundation of China (No. 20175018), the Natural Science Foundation of Guangxi Province (No. 2014016) and the Natural Science Foundation of Education Department of Guangxi Province.

The absorption spectrum was performed on a U-3400 spectrophotometer (Hitachi, Japan) or a RCT-560 spectrophotometer (Shanghai, China). A model PHS-10A meter (Science Apparatus Factory of Xiaoshan, China) was used to measure pH values of the solutions.

Reagents

A stock solution of HSA (2.0 mg/mL) (Beijing Branch of Sino-American Biotechnology Company) was prepared by directly dissolving HSA in doubly distilled water, stored at 0—4 ℃. Its working concentration was 10 μg/mL. A $K_3[Fe(CN)_6]$ stock solution (1.0×10^{-2} mol/L) was prepared by dissolving $K_3[Fe(CN)_6]$ in EDTA solution (2.5×10^{-2} mol/L). The working solution of $K_3[Fe(CN)_6]$ was obtained by diluting the stock solution to 1.0×10^{-4} mol/L with water. All chemicals were of analytical grade or the top grade commercially available. Water used was doubly distilled.

Preparation of synthetic samples

According to the tolerances of foreign substances, interfering components were added in an appropriate of HSA standard solution to make up synthetic samples. To test the practicability of the method, three samples were constructed.

Standard procedure

Certain volume of HSA solution or sample solution, 2.0 mL of HCl solution (1.0×10^{-2} mol/L) and 2.0 mL of $[Fe(CN)_6]^{3-}$ solution (4.0×10^{-4} mol/L) were orderly added into a 10-mL test tube. Then the mixture was diluted to 10 mL with water and shaken well. Twenty minutes later, the resonance scattering spectrum and the intensity of resonance scattering at 351 nm were measured on a RF-540 fluorospectrophotometer. The resonance scattering spectrum was obtained by synchronous scattering on the RF-540 fluorospectrophotometer from 200 nm to 1000 nm (namely, $\Delta\lambda = 0$ nm). The resonance scattering intensity I_{RS} of the HSA system and the I_0 for the blank solution were measured by fixing the excitation wavelength and the emission wavelength at 351 nm. Both excitation and emission slits were 10 nm. The set of the fluorescence spectrophotometer was at low sensitivity. The value $\Delta I = I_{RS} - I_0$ was calculated.

Results and discussion

Spectral characteristics

The intensities of the $[Fe(CN)_6]^{3-}$-EDTA-HCl system and the HSA solution are rather weak and the two resonance scattering curves nearly overlap (curves A and B, in Fig. 1), which indicates the HSA molecules and the mixture of $[Fe(CN)_6]^{3-}$-EDTA-HCl can not cause obvious scattering; while HSA solution mixes with $[Fe(CN)_6]^{3-}$-EDTA-HCl, the resonance intensity obviously enhances (curve C in Fig. 1), which indicates that negatively charged ion $[Fe(CN)_6]^{3-}$ probably combines with positive charge HSA (isoelectric point, pH 4.7) to ion-association complex by intermolecular force (mainly by electrostatic attraction force). That is to say, a number of $[Fe(CN)_6]^{3-}$ molecules stack on a HSA macromolecule. Then theion association complexes further aggregate to supramolecules or particles in bigger size. And a clear interface between the supramolecules and water phase is formation. The reactions of $[Fe(CN)_6]^{3-}$ are indicated as in Eqs. (1) and (2).

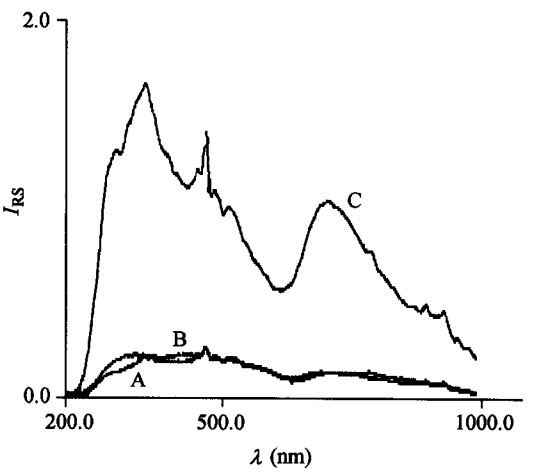

Fig. 1 Resonance scattering spectra. A: $[Fe(CN)_6]^{3-}$ (8.0×10^{-5} mol/L)-HCl (2.0×10^{-3} mol/L)-EDTA (2.0×10^{-4} mol/L); B: HSA (2 μg/mL); C: $[Fe(CN)_6]^{3-}$ (8.0×10^{-5} mol/L)-HCl (2.0×10^{-3} mol/L)-HSA (2 μg/mL)-EDTA (2.0×10^{-4} mol/L).

$$n[Fe(CN)_6]^{3-} + HSA^{m+} = [Fe(CN)_6]_n^{3-}HSA^{m+} \quad (1)$$

$$k[Fe(CN)_6]_n^{3-}HSA^{m+} = \{[Fe(CN)_6]_n^{3-}HSA^{m+}\}_k \quad (2)$$

The strongest resonance scattering peak of the $\{[Fe(CN)_6]^{3-}_n HSA^{m+}\}_k$ system is located at 351 nm (Fig. 1C) corresponding to the absorption valley of curve A in Fig. 2. This is owing to the reason that the $[Fe(CN)_6]^{3-}$ molecules absorb incident and scattering light simultaneously. According to the intensity function of resonance scattering, $I_{RS} = KI_K f(d) f(\lambda) c$, here the d represents diameter, the valid incident light intensity I_K will decrease as there is molecule absorption.[10,11] Several types of absorption were divided according to the following results of the photons absorbed. The photons absorbed may be transformed into heat energy, such as the absorption of $[Fe(CN)_6]^{3-}$, may emit in Stock or anti-Stock fluorescence or phosphorescence, and cause the photochemical reaction *etc*. If the photons absorbed do not emit in irradiation or emit at the different λ_{ex}, this type of absorption is called inelastic absorption by us owing to existence of energy lose. It causes the decrease of resonance scattering intensity. For example, the I_{RS} decreases when the concentration of $[Fe(CN)_6]^{3-}$ is more than 1.0×10^{-4} mol/L, owing to the existence of associated $[Fe(CN)_6]^{3-}$ in the system. Therefore, a signal of the resonance scattering weaker than the I_K of the lamp-house was obtained at the maximum absorbance wavelength of molecule.[11] For nanoparticle system, the photons absorbed may emit at the same wavelength λ_{ex} but the emission efficiency is less than 100%. The $[Fe(CN)_6]^{3-}$ HSA^{m+} nanoparticle system is one of the examples. In other case, for example, molecular resonance fluorescence system, the fluorescence wavelength λ_{em} is equal to the λ_{ex}. And the resonance fluorescence efficiency is commonly less than 100%. Such type of absorption is called quasi-elastic absorption, which produces resonance scattering. The resonance scattering peak at 700 nm in Fig. 1 is a 1/2 fraction of frequency resonance scattering peak of 351 nm,[12] owing to the interaction both the photon, the grating and the $\{[Fe(CN)_6]^{3-n}HSA^{m+}\}_k$ nanoparticle. The indent distance of grating is about 667 nm (= 1 mm/1500 line). The grating can be regarded as two-dimension assembly of particles. The particle solutions can be regarded as three-dimension grating, like NaCl crystal grating in X-ray diffraction. And the nanoparticle of $\{[Fe(CN)_6]^{3-}_n HSA^{m+}\}_k$ locates at fixed position when the photon interacts with the particle, owing to the free motion speed of the particle in solution is much less than the velocity of the excited photon. The peak at 470 nm in Fig. 1 is owing to the uneven-distribution emission intensity of Xe lamp.[13] In short, there are two important factors including inelastic absorption and lamp-house to produce resonance scattering peak.

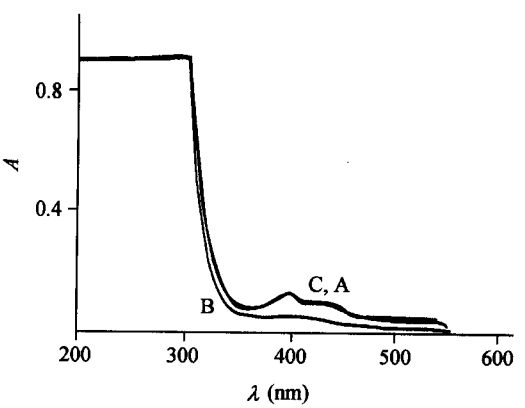

Fig. 2 Absorption spectra (conditions are the same as in Fig. 1).

Effect of the conditions

Fig. 3 shows that the ΔI reaches its maximum and has few variations at pH 2.64—3.09. A citric acid-NaOH buffer had been tested to adjust the solution acidity to pH 2.64—3.09. But its ΔI value is smaller than that in the medium of weak hydrochloric acid. So a 2.0×10^{-3} mol/L HCl medium (pH about 2.70) was chosen as the optimum medium.

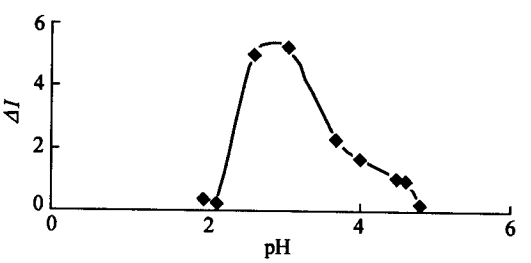

Fig. 3 Effect of pH on scattering intensity [HAS (0.3 μg/mL)-$[Fe(CN)_6]^{3-}$ (8.0×10^{-5} mol/L) ordinate scale 5].

Effect of $[Fe(CN)_6]^{3-}$ concentration on the scattering intensity is shown in Fig. 4. It can be seen that, when $[Fe(CN)_6]^{3-}$ concentration is low, ΔI is also weak, which may imply un-complete combination between HSA and $[Fe(CN)_6]^{3-}$. However, if the $[Fe(CN)_6]^{3-}$ concentration is more than 10×10^{-5} mol/L, the scattering intensity gets weaker because of the existence of inelastic absorption for the unreaction $[Fe(CN)_6]^{3-}$

molecules, that is, there are the associated $[Fe(CN)_6]^{3-}$ molecules in the system. When $[Fe(CN)_6]^{3-}$ concentration is in the range of 7.0×10^{-5}—10.0×10^{-5} mol/L, the ΔI value reaches its maximum and is stable. Therefore, $[Fe(CN)_6]^{3-}$ concentration at 8.0×10^{-5} mol/L was chosen for assay. When HSA concentration is 1.4×10^{-8} mol/L, the $[Fe(CN)_6]^{3-}$ concentration reaching maximum ΔI value is 7.0×10^{-5} mol/L. The mean combination number of $[Fe(CN)_6]^{3-}$ to one HSA is about 5.0×10^3 ($n = 7.0 \times 10^{-5}$ mol/L/1.4×10^{-8} mol/L). It is known that HSAs have 58 of Lys, 23 of His and 16 of Arg. Its total number of basic ammonia acid is 98.[14,15] The mean combination number determined is much more than the total number of basic ammonia acid in HSA. This shows that the interaction between HSA and $[Fe(CN)_6]^{3-}$ is complexity. There may be multi-layer stacking of $[Fe(CN)_6]^{3-}$ on HSA.

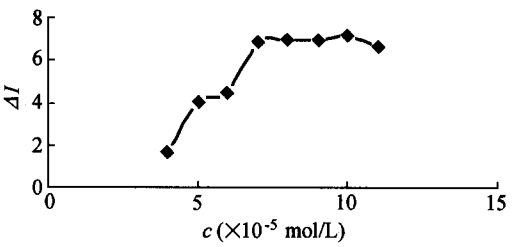

Fig. 4 Effect of $[Fe(CN)_6]^{3-}$ on $I_{351\ nm}$[HCl (2.0×10^{-3} mol/L)-HSA (1 μg/mL) ordinate scale 4].

The results of the effect of ionic strength (NaCl concentration, c_{NaCl}) show that the ΔI decreases gradually with the increase of the NaCl concentration. This is due to the complete binding effect of Cl^- to HSA and the shielding effect of NaCl to the charges of HSA and $[Fe(CN)_6]^{3-}$, which leads to the decrease of binding ability between HSA and $[Fe\text{-}(CN)_6]^{3-}$.

The effect of both EDTA and addition sequence of reagents on scattering was also investigated. The scattering intensity of reagent blank (I_0) was found to decrease greatly and be stable for a long time when EDTA was added in experimental system. The probable reason is that EDTA coordinates with trace metal ion in reagents to form complex, which avoids metal ions such as Fe^{2+} or Cu^{2+}, combining with $[Fe(CN)_6]^{3-}$ to un-dissolved particles which produce light scattering. No matter that reagent addition sequence, their resonance scattering intensities approach approximate value in 20 min after the last mix and are stable for 1 h or so. Therefore, in experimental system 2.0×10^{-4} mol/L EDTA is suggested to be used and the measurement time is chosen in 20 min after the last mix.

Effect of surfactants on the scattering intensity

Effects of three surfactants [cetyltrimethylammonium bromide (CTAB), sodium dodecly sulfate(SDS) and Triton X-100] on the assay were tested. Little amount of CTAB can enhance the scattering intensity of reagent blank. It can be inferred that $[Fe(CN)_6]^{3-}$ binds with CTAB by electrostatic attraction to ion-association complex which leads to the increase of scattering intensity; while there are proteins in experimental system, CTAB can not bind with HSA because of electrostatic repulsion. When the CTAB concentration is higher than 3 μg/mL, the scattering intensity of experimental system decreases sharply. As far as the mechanism of reaction is concerned, the combination of HSA with $[Fe(CN)_6]^{3-}$ is the same as that of CTAB with $[Fe(CN)_6]^{3-}$.

The results show that the variation of SDS concentration has no influence on scattering intensity in the absence of proteins, which indicates SDS can not combine with $[Fe(CN)_6]^{3-}$ because of electrostatic repulsion and SDS itself has no contribution to light-scattering. In the presence of HSA, the variation of SDS concentration also has no obvious influence on scattering intensity. The probable reason is that the binding force between $[Fe(CN)_6]^{3-}$ and HSA is stronger than that between SDS and HSA. So the increase of SDS concentration does not enhance the scattering intensity of experimental system. With increasing SDS concentration, the aggregations may be formed among SDS molecules which weakens the effect of scattering.

The relationship between Triton x-100 concentration and scattering intensity shows that whether in presence of HSA or not, Triton X-100 concentration has no obvious effect on scattering intensity. It can be inferred that Triton X-100 can not combine with $[Fe(CN)_6]^{3-}$ to ion-association complex, and the complex formed by HAS and $[Fe(CN)_6]^{3-}$ can no longer combine with Triton x-100 by intermolecular force. The effects of the above three surfactants on reaction system have their respective features. It is relevant to effect reaction mechanism. On the whole, the three surfactants have no enhanced sensitivity for HSA assay.

Interferences of coexisting foreign substances

The influences of foreign coexisting substances such as amino acids, metal ions were tested. The results are presented in Table 1. From Table 1, it can be seen that amino acids have no effect on scattering intensity of HSA-$[Fe(CN)_6]^{3-}$ system. Common metal anions except for Fe^{3+} also have no obvious influence on the scattering intensity of HSA-$[Fe(CN)_6]^{3-}$ system. Comparatively larger interference on the HSA assay by Fe^{3+} was observed. It maybe owing to the formation of metal complex anion FeY^{3-} complexes,[14] causing the enhancement of resonance scattering intensity.

Calibration curve

According to the above standard procedures, different concentration solutions of HSA were used to construct the calibration of HSA. The concentration of HSA (c μg/mL) in the range of 0—12 μg/mL is linear to the resonance scattering intensity. The linear regression equation is $\Delta I = 0.21 + 6.12c$ and its correlation coefficient 0.9991. The relative standard deviations (RSD) for five measurements of HSA (1 μg/mL) and HSA solution (0.5 μg/mL) are 2.4% and 2.5%, respectively. The determination limit of this method is 0.1 μg/mL. The detection limit (3σ) is 30 ng/mL, higher than that of Coomassie brilliant blue protein assay.

Analytical application

The content of HSA in synthetic sample was determined by this RSS method, and the recovery of HSA is in the range of 89.7%—96.2%. The determination results for synthetic samples are shown in Table 2. The protein content in human serum samples has been determined by this RSS method after the dilution of 5000 times with water. The results are shown in Table 3, which are in agreement with those of coomassie brilliant blue-250 spectrophotometry (CBB-250).

Table 1 Tolerance limit of foreign substances

Substance	Concentration (μg/mL)	Relative error in I_{RS} (%)	Substance	Concentration ($\times 10^{-5}$ mol/L)	Relative error in I_{RS} (%)
L-Tyr	50	−2.4	Fe(II)(sulfate)	5.0	+0.4
L-Trp	50	−3.8	Mn(II)(sulfate)	10	+0.23
L-Asp	20	+4.8	Zn(II)(sulfate)	10	−0.1
L-Cys	20	+2.4	Cu(II)(sulfate)	10	−0.05
L-His	20	+4.8	Mg(II)(sulfate)	10	+0.11
L-Met	20	+4.0	Fe(III)(chloride)	1.0	+9.9
L-Lys	100	+0.6	Na(I)(chloride)	5.0	+0.21
L-Gly	50	+1.8	Pb(II)(acetate)	10	+0.11
L-Thr	50	−1.2	Ba(II)(chloride)	5.0	−0.05
L-Val	50	−2.4	K(I)(iodide)	5.0	+0.4
			$[Fe(CN)_6]^{4-}$	8.0	+1.3

Table 2 Determination results for synthetic samples

Sample	Components of synthetic sample ($\times 10^{-5}$ mol/L)	Found ($n=5$, μg/mL)	Recovery (%) ($n=5$)	RSD (%) ($n=5$)
1	$FeCl_3$(5)-NaCl(5)-*L*-Trp[a](10)-*L*-Asp[a](10)-HSA[a](3)	2.60	89.7	0.42
2	$MgSO_4$(5)-*L*-Tyr[a](10)-*L*-Met[a](10)-*L*-Lys[a](10)-HSA[a](3)	2.89	96.2	0.50
3	$MgSO_4$(5)-*L*-Lys[a](10)-*L*-Gly[a](10)-*L*-Thr[a](10)-HSA[a](3)	2.60	94.7	0.38

[a] The unit of concentration is μg/mL.

Table 3 Analytical results for human serum samples

Sample	RSS method of $K_3[Fe(CN)_6]$			CBB-250 method	
	Concentration determined ($\mu g/mL$)	Content (mg/mL)	RSD (%, $n=5$)	Content (mg/mL)	RSD (%, $n=5$)
1	1.30	65.0	2.5	70.4	2.3
2	1.24	62.0	3.3	63.6	2.7
3	1.35	63.5	3.1	64.6	3.2

Mechanism of enhanced resonance scattering

Some references have been pointed out that the Rayleigh scattering formula is suitable to the no absorption molecular scattering or the scattering of small particle in molecular size, and not for the colloid, frog, microemulsion and so on.[15-18] The molecular scattering is based on the fluctuation theory of density in physics. The Mie scattering formula is suitable to the spherical particle, and is very complexity. We have known that the absorption of aqueous nanoparticle system is different from molecular absorption in spectrophotometric analysis, owing to the interface formation and resonance scattering, such as metal nanoparticle in liquid, some supramolecule in water, bubble, frog and microemulsion *etc*. It was demonstrated that the ultimate cause is the interface formation of nanoparticle and supramolecule in large size, to enhance scattering signal.[19-24] In the absence of $K_3[Fe(CN)_6]$, HSA micromolecule produces very weak resonance light scattering, owing to the interface between HSA particle and water phase is not clear although HSA size (about 40 nm) is large. In the presence of $K_3[Fe(CN)_6]$, the HSA^{m+} and $[Fe(CN)_6]^{3-}$ combine into $[Fe(CN)_6]_n^{3-}$-HSA^{m+} by means of electrostatic force . The nanoparticle or supramolecular of $\{[Fe(CN)_6]_n^{3-}HSA^{m+}\}_k$ was formed by means of intermolecular forces (Fig. 5). The multilayer $[Fe(CN)_6]^{3-}$ molecules covers the surface of HSA micromolecule (Fig. 6). Of course, small nanoparticle of $\{[Fe(CN)_6]_n^{3-}HSA^{m+}\}_k$ may aggregate to big particle, owing to its high surface energy.[21] The size of $\{[Fe\text{-}(CN)_6]_n^{3-}HSA^{m+}\}_k$ nanoparticle is in the range of 73—300 nm, and larger than that of HSA. The interface between $\{[Fe(CN)_6]_n^{3-}HSA^{m+}\}_k$ particle and water phase is more clear. So that it exhibits strong resonance scattering. No TEM of HSA was observed, owing to the low electron density of C, O, N and H. But Fe^{3+} has high electron density. And the TEM of $\{[Fe\text{-}(CN)_6]_n^{3-}HSA^{m+}\}_k$ particle was recorded as in Fig. 5.

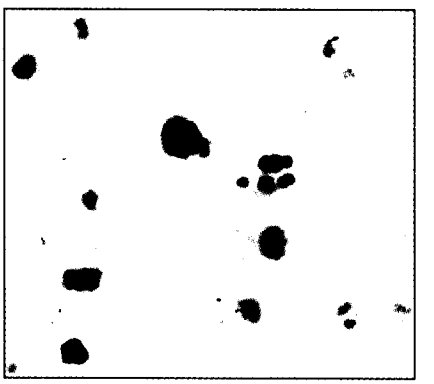

Fig. 5 TEM of $\{[Fe(CN)_6]_n^{3-}HSA^{m+}\}_k$ magnified 10000 times.

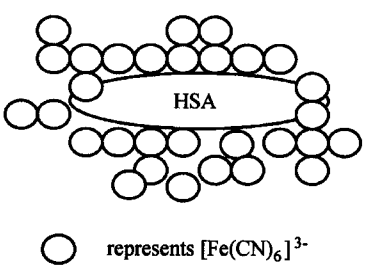

Fig. 6 Diagram of $\{[Fe(CN)_6]_n^{3-}HSA^{m+}\}_k$ nanoparticle.

Conclusion

The TEM demonstrates that there are $\{[Fe\text{-}(CN)_6]_n^{3-}HSA^{m+}\}_k$ nanoparticles or supramolecules. The formation of nanoparticle in big size results to enhance signal of resonance scattering. The resonance scattering peak at 351 nm is produced mainly by the Xe lamp and inelastic absorption of $[Fe(CN)_6]^{3-}$ molecule or surface $[Fe(CN)_6]^{3-}$ molecule on the nanoparticle. There is no quanta colored effect resulting to a characteristic resonance scattering peak.[19,21,23] A new resonance scattering spectral method for the determination of HSA in human serum has been proposed with satisfactory results.

References

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

2 Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248.

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

4 Liu, S.-P.; Liu, Q. *Anal. Sci.* **2001**, *17*, 239.

5 Liu, S.-P.; Yang, R.; Liu, Q. *Anal. Sci.* **2001**, *17*, 243.

6 Ma, C.-Q.; Li, K.-A.; Tong, S.-Y. *Fresenius' J. Anal. Chem.* **1997**, *357*, 915.

7 Wei, Y.-J.; Li, K.-A.; Tong, S.-Y. *Acta Chim. Sinica* **1998**, *56*, 290 (in Chinese).

8 Tao, G.; Li, K.-A.; Tong, S.-Y. *Anal. Chim. Acta* **1999**, *389*, 319.

9 Feng, P.; Hu, X.-L.; Huang, C.-Z. *Anal. Lett.* **1999**, *32*, 1323.

10 Jiang, Z.-L.; Li, F.; Li, T.-S. *Anal. Testing Technique and Instruments*, **2000**, *6*, 150 (in Chinese).

11 Jiang, Z.-L.; Li, F.; Liu, Q.-Y. *Chin. J. Inorg. Chem.* **2001**, *16*, 355 (in Chinese).

12 Jiang, Z.-L. *Acta Photonica Sinica* **2001**, *30*, 460 (in Chinese).

13 Jiang, Z.-L.; Li, F. *Guangxi Sciences* **2001**, *8*(2), 93 (in Chinese).

14 Wang, K. *Bioinorganic Chemistry*, Qinghua University Press, Beijing, **1988**, p. 216 (in Chinese).

15 Liang, H.; Shen, X.-C.; Jiang, Z.-L.; He, X.-W.; Sheng, P. W. *Sci. China, Ser. B* **2000**, *43*, 460.

16 Zhao, G.-H.; Zhong, X.-H. *Optics*, Beijing University Press, Beijing, **1983**, p. 251 (in Chinese).

17 Zhou, S.-Z. *Optical Phenomena of Atmosphere*, Shanghai Science and Technology Press, Shanghai, **1982**, p. 23 (in Chinese).

18 Chen, S.-C.; Chen, C. M. *Meteorology*, Chinese Agricultural Press, Beijing, **1981**, p. 129 (in Chinese).

19 Jiang, Z.-L.; Li, F.; Li, T.-S. *Chem. J. Chin. Univ.* **2000**, *21*, 1488 (in Chinese).

20 Xie, J.-Y.; Jiang, Z.-L. *Acta Phys.-Chem. Sin.* **2001**, *17*, 406 (in Chinese).

21 Jiang, Z.-L.; Feng, Z.-W.; Li, F.; Li, T.-S.; Zheng, F.-X.; Xie, J.-Y.; Yi, X.-H. *Sci. China, Ser. B* **2001**, *44*, 175.

22 Jiang, Z.-L.; Feng, Z.-W. *Chem. J. on Internet* **2001**, *03*(*4*), 017ne.

23 Jiang, Z.-L.; Li, F.; Li, T.-S. *Acta Chim. Sin.* **2001**, *59*, 438 (in Chinese).

24 Jiang, Z.-L.; Feng, Z.-W. *Chin. Chem. Lett.* **2001**, *13*, 443.

(E0112056 SONG, J. P.; DONG, L. J.)