ISSN : 0974 - 7524 Volume 10 Issue 4

Physical CHEMISTRY
An Indian Journal

Full Paper

PCAIJ, 10(4), 2015 [142-148]

Investgation on the reaction mechanism of lysozyme with metronidazole by improved spectroscopy

Rong Han, Baosheng Liu*, Gaixia Li, Qiuju Zhang

Key Laboratory of Analytical Science and Technology of Hebei Province,College of Chemistry & Environmental Science, Hebei University, Baoding 071002, Hebei Province, P. R. China, (CHINA)

E-mail: lbs@hbu.edu.cn

ABSTRACT

To resolve the deficiencies in the interaction analysis by classical fluorescence spectroscopy with protein as research object, elastic scattering fluorescence spectroscopy with drugs as research object had been applied to study the interaction mechanism between metronidazole (MET) and lysozyme(LYSO) under human physiological conditions at different temperatures. The results clearly demonstrated that the interaction mechanism obtained by elastic scattering fluorescence spectroscopy in MET-LYSO system was consistent with fluorescence spectroscopy that LYSO was used as investigated object. The binding constant (K_a) that obtained by elastic scattering fluorescence spectrometry were much larger than the one obtained by classical fluorescence spectroscopyÿwhich indicated that reaction information was more accurate and comprehensive when regarding the drug as the investigated object. At lastÿthe scientificalness of the method based on elastic scattering fluorescence spectroscopy was verified by UV-visible absorption spectroscopy.

KEYWORDS

Lysozyme;
Metronidazole;
Elastic scattering fluorescence spectrometry;
UV-visible absorption spectroscopy;
Classical fluorescence spectroscopy;
Reaction;
Mechanism.

INTRODUCTION

The classical fluorescence spectroscopy[1] is used to investagate the interaction mechanism of drugs and proteins, by analysising the change of fluorescence intensity of protein at the maximum emission wavelength before and after adding the drugs. The binding constants, binding sites, type of interaction force and cooperative binding between drugs and proteins are obtained from that method. Elastic light scattering[2] is a kind of light scattering, which the radiation light wavelength is the same with incident light wavelength. In classical fluorescence spectroscopy, the dominant ûuorophores of LYSO[3] is tryptophan residues-62. Classical fluorescence spectroscopy does not reflect interaction of the other non-fluorescence-emitting residues with drugs, so using classical fluorescence spectroscopy can't express interaction information roundly. To resolve the deficiencies, the improved spectroscopy with drug as detection object was investigated in this article.

Lysozyme(LYSO)[4] is an antimicrobial protein-

ase, consists of 129 amino acid residues including six tryptophan (Trp) and three tyrosine (Tyr) residues. The six Trp residues[5] are located at the substrate binding sites, out of which two are in the hydrophobic matrix box, while the lone Trp residue is separated from the others. Among the six Trp residues[6], Trp62 and Trp108 are considered to be the most dominant ûuorophores in LYSO. It has many physiological and pharmaceutical functions[7], such as, antibacterial,antivirus and detumescence and it can also improve the human blood circulation and enhance the human immunity. Another important function of LYSO[8] is its ability to carry drugs. It can cure some illness via the binding with active compounds. The effectiveness of drugs depends on their binding ability.

Metronidazole[9] (MET) (the structure shown in Figure 1) belongs to the nitroimidazole group. It is employed in both human and veterinary medicine to treat diseases caused by anaerobic bacteria(Bacteroides, Fusobacterium, Campylobacter, Clostridium) and protozoa (Trichomonas, Treponema, Histomonas). In the present work, the binding mechanism between LYSO and MET is investigated by classical fluorescence method, elastic scattering fluorescence spectroscopy and UV-visible absorption spectroscopy, respectively. The binding constant obtained from elastic scattering fluorescence spectroscopy with drug as investigated object were larger than the one obtained from classical fluorescence spectroscopy with protein as investigated object at the same temperature, which indicated that the improved spectroscopy can express interaction information more accurate and was used by UV-vis spectroscopy to verify its rationality.

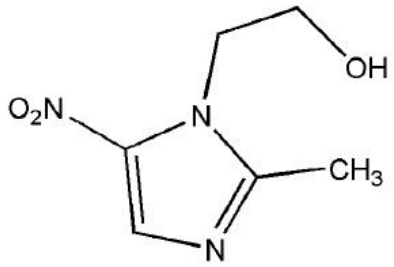

Figure 1 : Chemical structure of Metronidazole

EXPERIMENTAL

Apparatus

All ûuorescence spectra were recorded with a Shimadzu RF-5301PC spectroûuorophotometer. Absorption was measured with an UV/vis recording spectrophotometer (UV-265, Shimadzu,Japan). All pH measurements were carried out with a pHS-3C precision acidity meter (Leici, Shanghai, China). All temperatures were controlled by a CS501 superheated water bath (Nantong Science Instrument Factory).

Materials

Metronidazole(MET) was purchased from Yabao Pharmaceutical Group Co.,Ltd. Monitor(the purity grade inferior 99%). Lysozyme(LYSO) was purchased from Sigma Co (the purity grade inferior 99%). Stock solutions of LYSO (2.0×10^{-5}mol·L^{-1}) and MET (1.0×10^{-3}mol·L^{-1}) were prepared. All the stock solutions were further diluted as working solutions prior to use. Tris–HCl buffer solution containing NaCl (0.15 mol·L^{-1}) was used to keep the pH of the solution at 7.4. NaCl solution was used to maintain the ionic strength of the solution. All other reagents were of analytical grade, and all aqueous solutions were prepared with newly double-distilled water and stored at 277K.

Procedures

Classical fluorescence spectroscopy measurements.

1.0mL of pH=7.40 Tris-HCl, 0.2mL of LYSO solution(2.0×10^{-5} mol·L^{-1})and different concentrations of MET were added into 10 mL colorimetric tube successively. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static at different temperatures (298, 310and 318 K). The excitation wavelength for LYSO was 280nm with the excitation and emission slit widths set at 5nm. The solution was subsequently scanned on the fluorophotometer and determined the fluorescent intensity at 341nm.

Elastic scattering fluorescence spectroscopy measurements

Full Paper

1.0mL of pH=7.40 Tris-HCl, 1.0 mL of MET solution (2.0×10^{-4} mol·L^{-1}) and different concentrations of LYSO were added into 10 mL colorimetric tube successively. The samples were diluted to scaled volume with water, mixed thoroughly by shaking, and kept static at different temperatures (298, 310and 318 K). The fluorescence spectra were measured ($\Delta\lambda$ at 0 nm and emission wavelengths of 230-660 nm). The widths of both excitation and emission slit were set to 5 nm. The fluorescent intensity I at 278 nm was recorded.

UV-vis absorption measurements.

Solution preparation was the same with elastic scattering fluorescence spectroscopy measurements, with corresponding concentration of LYSO solution as the reference. The UV-visible absorption spectra of MET in the presence and absence of LYSO were scanned with 1 cm quartz cells in the range from 190 to 430nm and the absorption intensity A at the maximum absorption peak was recorded.

RESULT AND DISCUSSION

Fluorescence quenching spectra of LYSO–MET system

The fluorescence spectra of LYSO-MET system were shown in Figure 2. As shown in Figure 2, the fluorescence intensity of LYSO decreased gradually with the addition of MET. Meanwhile, a red shift of emission wavelength from 339 to 360nm was observed. This result showed that MET could quench the intrinsic fluorescence of LYSO strongly and the quenching of LYSO fluorescence by MET was due to the formation of the LYSO–MET ground-state complex[10].

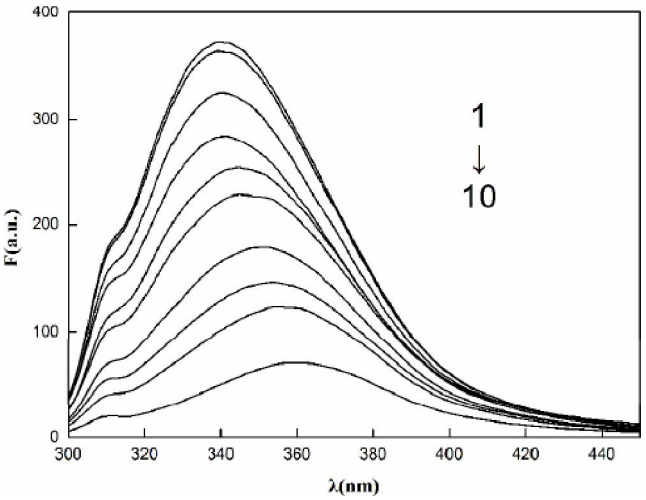

Figure 2 : Fluorescence emission spectra of LYSO-MET(T=298K), λ_{ex}=280nm;C_{LYSO}=4.0×10^{-7} mol·L^{-1}, 1~10: C_{MET}= (0, 0.2, 1.0, 2.0, 4.0, 6.0, 8.0, 10, 13, 18)×10^{-5} mol·L^{-1}

In order to confirm the quenching mechanism, the fluorescence quenching data were analyzed by the Stern–Volmer equation[11]

$$F_0 / F = 1 + K_q \tau_0 [L] = 1 + K_{sv} [L] \quad (1)$$

Where F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively.τ_0 is the average lifetime of fluorescence without quencher and is 10^{-8} s. K_{sv} is the Stern–Volmer quenching constant. K_q is the quenching rate constant of biomolecule, and [L] is the concentration of the quencher. Based on the linear fit plot of F_0/F versus [L], the K_q values can be obtained. The calculated results were shown in TABLE 1. From TABLE 1, it can be seen that the values of K_{sv} decreased with increasing temperature for LYSO-MET systems, which indicated that the probable quenching mechanism of the interaction between LYSO and MET was a static process[12]. The value of K_q were much greater than the maximum scatter collision quenching constant of various quenchers (2×10^{10} L·mol^{-1}·s^{-1}), which also suggested that the quenching was not initiated by dynamic collision but complex formation between LYSO and MET[13].

For static quenching process, the relationship between the fluorescence intensity and the concentration of quencher could be usually described by equation (3) to obtain the binding constant (K_a) and the number of binging sites (n) in most of paper[14]:

$$\log\left(\frac{F_0 - F}{F}\right) = n\log K_a + n\log\left\{[D_t] - n\frac{F_0 - F}{F_0}[B_t]\right\} \quad (2)$$

where [Dt] and [Bt] are the total concentrations of MET and LYSO, respectively. On the assumption that n in the bracket is equal to 1, the curve of log(F_0 -F)/F versus log{[D_t]-[B_t](F_0 -F)/F_0}is drawn and fitted linearly, then the value of n can be obtained from the slope of the plot. If the n value obtained is not equal to 1, then it is substituted into the bracket and the curve of log(F_0-F)/F versus log{[D_t]-[B_t](F_0 -F)/F_0} is drawn again. The above process is re-

TABLE 1 Quenching reactive parameters of MET and LYSO at different temperatures

T/(K)	K_q /(L·mol^{-1}·s^{-1})	K_{sv} / (L·mol^{-1})	r_1	K_a /(L·mol^{-1})	n	r_2
298	2.01×10^{12}	2.01×10^{4}	0.9942	1.77×10^{4}	1.06	0.9961
310	1.96×10^{12}	1.96×10^{4}	0.9956	1.70×10^{4}	1.02	0.9973
318	1.93×10^{12}	1.93×10^{4}	0.9947	1.35×10^{4}	0.99	0.9915

K_q is the quenching rate constant; K_a is the binding constant; n is the number of binding site; r_1 is the linear relative coefficient of F_0/F~$[L]$; r_2 is the linear relative coefficient of $\log(F_0-F)/F$~$\log\{[D_t]-n[B_t](F_0-F)/F_0\}$.

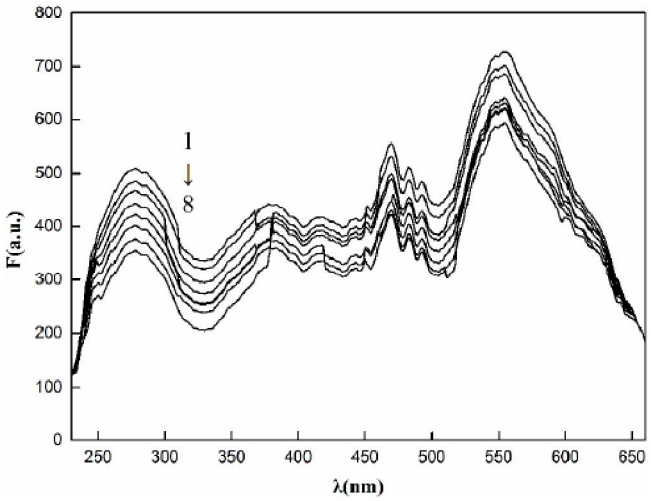

Figure 3 : Elastic scattering fluorescence spectra of LYSO-MET system (T = 298K); C_{MET} = 2.0×10^{-5} mol·L^{-1}, 1~8 C_{LYSO} = (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0)×10^{-7} mol·L^{-1}

peated again and again till n obtained is only a single value or a circulating value. Based on the n obtained, the binding constant K_a can be also obtained. In the work, a calculation program was developed. The calculation process can be finished with calculator based on the simple program, and the calculating results can be obtained by inputting F, $[D_t]$ and $[B_t]$. The calculated results were shown in TABLE 1. As seen in TABLE 1, the values of n were approximately equal to 1 at different temperatures, indicating there is one binding site of MET to LYSO. Meanwhile, the binding constants K_a decreased with increasing temperature implied the complex of MET–LYSO became less stable at higher temperature, which further evidenced that the fluorescence quenching was a static quenching process[15].

Elastic scattering fluorescence spectroscopy studies

The scattering fluorescence spectra of LYSO-MET system were shown in Figure 3. As shown in Figure 3, with gradual addition of LYSO to MET solution, the scattering intensity of the peak at 278 nm decreased. The results indicated that the interaction between LYSO and MET led to the formation of a complex between drug and protein[16]. According to the eqns. 1, 2, the calculated results were shown in TABLE 2. From TABLE 2, it can be seen that the number of binging sites (n) were all equal to 1. Meanwhile, K_{a1} and K_{sv1} were all decreased with increasing temperature in LYSO-MET systems, further suggested that the quenching was a static process, which were consistent with the results of classical fluorescence spectroscopy. This indicated that taking protein or drug as the studied object, although experimental methods were different, obtationed the same interaction mechanism between drugs and protein. In addition, the K_{a1} values of elastic scattering fluorescence spectroscopy with drug as detection object were much greater than the K_a values of classical fluorescence spectroscopy with protein as detection object at the same temperature, which showed that not only the Trp62 of LYSO participated in the interaction between LYSO and MET, but the other amino acid residues also took part in the LYSO-MET systems[17]. Furthermore, compared with classical fluorescence spectroscopy with protein as research object, treating drugs as investigated object can give more comprehensive and more accurate of the interaction information between protein and drugs.

UV-visible spectra studies

The binding constant K_b of protein and drug can be calculated from the following eqn. 3[18]:

$$(A_0 - A)^{-1} = A_0^{-1} + K_b^{-1}A_0^{-1}[L]^{-1} \quad (3)$$

where, A_0 and A are the absorption values in the absence and presence of ligand, respectively. And [L] is the concentration of the ligand. The UV-visible absorption spectra of MET in the absence and presence of LYSO were shown in Figure 4. As shown in Figure 4, with gradual addition of LYSO to MET

Full Paper

TABLE 2 : Quenching reactive parameters of MET and LYSO at different temperatures

T/(K)	K_{q1} /(L·mol^{-1}·s^{-1})	K_{sv1} /(L·mol^{-1})	r_3	K_{a1} /(L·mol^{-1})	n	r_4
298	5.01×10^{13}	5.01×10^{5}	0.9933	3.28×10^{6}	1.13	0.9952
310	4.03×10^{13}	4.03×10^{5}	0.9958	2.45×10^{6}	1.12	0.9953
318	2.93×10^{13}	2.93×10^{5}	0.9931	1.89×10^{6}	1.06	0.9948

r_3 is the linear relative coefficient of F_0/F~$[L]$;r_4 is the linear relative coefficient of $\log\{(F_0-F)/F$~$\log\{[D_t]-n[B_t](F_0-F)/F_0\}$.

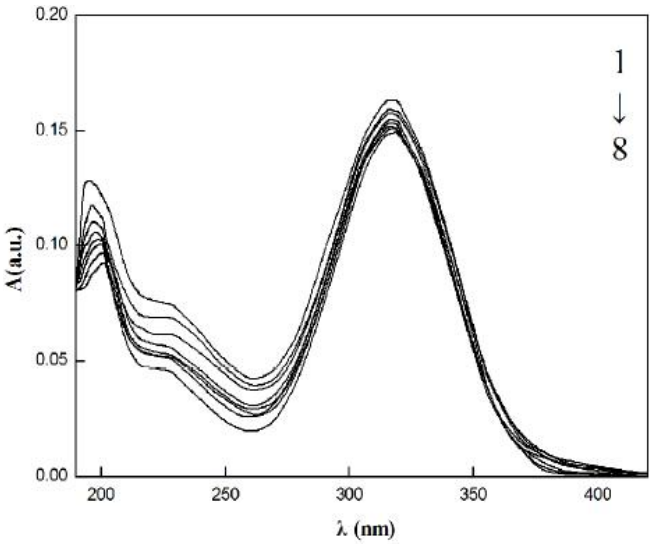

Figure 4 : Absorption spectra of LYSO-MET system (T = 298K); C_{MET} = 2.0×10^{-5} mol·L^{-1}, 1~8 C_{LYSO} = (0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0)×10^{-7} mol·L^{-1}.

solution, the absorbance intensity of LYSO-MET systems decreased gradually, which indicated that the probable quenching mechanism of the interaction between LYSO and MET was a static process[19]. Based on the linear regression plot of $(A_0-A)^{-1}$versus $[L]^{-1}$, the K_b values can be obtained. The calculated results were shown in TABLE 3. As seen in TABLE 3, the binding constant K_b decreased gradually with rising temperatures, which is consistent with the results from classical fluorescence method. In addition, the K_b values were much larger than K_a from the classical fluorescence spectroscopy and close to K_{a1} obtained by the elastic scattering fluorescence spectroscopy at the same temperature. This indicated that UV-visible absorption spectroscopy have verified the scientificalness of the method based on elastic scattering fluorescence spectrometry, and treating the drug as detection objects can give more complete and more accurate interaction information of protein and drugs. In this experiment, the differences of binding constants may be caused by the intrinsic differences between elastic scattering fluorescence method and UV-visible method.

Type of interaction force in LYSO–MET systems

Generally, the interaction forces between the small drug molecule and biological macromolecule include hydrogen bond, Van der Waal's force, electrostatic interactions and hydrophobic force[20]. The thermodynamic parameters can be calculated on the basis of the following equation[21]:

$$R\ln K = \Delta S - \Delta H / T \quad (4)$$

$$\Delta G = -RT\ln K = \Delta H - T\Delta S \quad (5)$$

When temperature varies in a small range, the ΔH could be considered as a constant[22]. According to the binding constants at different temperatures above (TABLE 10TABLE 2 and TABLE 3), the thermodynamic parameters were obtained conveniently. The calculated results were shown in TABLE 4. The negative value of ΔG clarified a spontaneous reaction between LYSO and MET. The negative value of ΔH and positive value of ΔS showed that MET mainly bound to LYSO by the electrostatic attraction[23]. Comparing to ΔS, ΔG obtained by classical fluorescence spectroscopy and elastic scattering fluorescence spectroscopy, showed that the LYSO-MET systems which regarded MET as investigated object was easier proceed.The thermodynamic parameters obtained by elastic scattering fluorescence spectroscopy and UV-visible absorption spectroscopy were consistent, which indicates that the elastic scattering fluorescence spectroscopy with drug as detection object to study the interaction mechanism of MET with LYSO is rationality.

Hill's coefficient of LYSO–MET system

In biochemistry, the binding of a ligand molecule at one site of a macromolecule often influences the affinity for other ligand molecules at additional sites. This is known as cooperative binding. Hill's coefficient provides a way to quantify this effect and is calculated graphically on the basis of the following

TABLE 3 : The binding constants of LYSO-MET system by UV-visible spectra at different temperatures

T/(K)	K_b/(L·mol^{-1})	Linear regression equation	r_5
298	1.78×10^6	$(A_0\text{-}A)^{-1} = 6.498+3.647\times10^{-6}[L]^{-1}$	0.9914
310	1.53×10^6	$(A_0\text{-}A)^{-1} = 6.012 + 3.929\times10^{-6}[L]^{-1}$	0.9981
318	1.16×10^6	$(A_0\text{-}A)^{-1} = 6.835+ 5.875\times10^{-6}[L]^{-1}$	0.9961

K_b is the binding constant; r_5 is the linear relative coefficient of $(A_0\text{-}A)^{-1}$~$[L]^{-1}$.

TABLE 4 : The thermodynamic parameters of LYSO-MET system at different temperatures

Method	T /(K)	K /(L·mol^{-1})	ΔH /(KJ·mol^{-1})	r_6	ΔS/(J·mol^{-1}·K^{-1})	ΔG /(KJ·mol^{-1})
Classical fluorescence spectroscopy	298	1.77×10^4	-9.92	0.9921	48.03	-24.23
	310	1.70×10^4			48.98	-25.10
	318	1.35×10^4			48.87	-25.14
Elastic scattering fluorescence spectroscopy	298	3.28×10^6	-21.43	0.9956	52.82	-37.17
	310	2.45×10^6			53.18	-37.92
	318	1.89×10^6			52.76	-38.21
UV absorption spectroscopy	298	1.78×10^6	-16.20	0.9935	65.29	-35.66
	310	1.53×10^6			66.14	-36.70
	318	1.16×10^6			65.15	-36.92

r_6 is the linear relative coefficient of R lnK ~ T^{-1}.

TABLE 5 : Hill coefficient of LYSO-MET systems at different temperatures

T / (K)	Classical fluorescence spectroscopy		Elastic scattering fluorescence spectroscopy		UV absorption spectroscopy	
	n_H	r_7	n_H	r_7	n_H	r_7
298	1.151	0.9982	1.202	0.9922	1.182	0.9970
310	1.143	0.9975	1.175	0.9936	1.158	0.9959
318	1.012	0.9936	1.104	0.9915	1.067	0.9981

r_7 is the linear relative coefficient of lg[Y/(1-Y)]~lg[L].

equation[24] :

$$\lg\frac{Y}{1-Y} = \lg K + n_H \lg[L] \quad (6)$$

Where Y is the fractional binding saturation; K is the binding constant and n_H is the Hill's coefficient. Hill's coefficient is greater than 1, which exhibits positive cooperativity. Conversely, Hill's coefficient is less than 1, which exhibits negative cooperativity. A coefficient of 1 indicates non-cooperative reaction.

$$\frac{Y}{1-Y} = \frac{Q}{Q_m - Q} \quad (7)$$

For fluorescence measurement:

$$Q = \frac{F_0 - F}{F_0} \quad (8)$$

For UV-vis absorption measurement:

$$Q = \frac{A_0 - A}{A_0} \quad (9)$$

Where $1/Q_m$=intercept of the plot 1/Q versus 1/[L]. Hill's coefficients were presented in TABLE 5. As seen in TABLE 5, the values of n_H were slightly more than 1 in the systems at different temperatures, which indicated positive cooperativeness in the LYSO-MET systems, but they were weak. In addition, the values of n_H were inversely correlated with increasing temperature, it was illustrated that the positive cooperativeness ability of MET or LYSO was decreased with the previous ligand gradually. It was also one of the reasons which led to the reduced binding constants with increasing temperature[25]. Comparing the values of n_H obtained by elastic scattering fluorescence and UV-vis absorption spectroscopy with drug as detection object and classical fluorescence spectroscopy with protein as detection object at the same temperature, showed that the positive cooperativeness ability obtained by improved

spectroscopy was more stronger than classical fluorescence spectroscopy, which was in accordance with "the K_b values were much larger than K_a from the classical fluorescence spectroscopy and close to K_{a1} obtained by the elastic scattering fluorescence spectroscopy at the same temperature".

CONCLUSIONS

The interaction between LYSO and MET under simulated physiological conditions was studied by using classical fluorescence spectroscopy and elastic scattering fluorescence spectroscopy and verified by UV-vis absorption spectroscopy in this article. Compared binding constants of the three methods, showed that the K_a values of classical fluorescence spectroscopy was smaller than improved spectroscopy and the later two values were approximate. That indicated that regarding drugs as studied object for improved spectroscopy can be more comprehensive and more accurate when expressing the interaction information between protein and drugs. In addition, besides the Trp62 of LYSO, the other amino acid residues could also participated in the interaction between LYSO and MET. The linear relative coefficient of linear regression equation of the improved method were above 0.99, suggesting that the elastic scattering fluorescence spectroscopy method is reasonable. The improved spectroscopy with drugs as studied object is investigated the interaction mechanism more accurately between drugs and protein, and is a challenge for classical fluorescence spectroscopy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of National Science Foundation of China (Grant no. 21375032) and Hebei Provincial Key Basic Research Program (Grant no. 10967126D).

REFERENCES

[1] X.N.Yan, B.S.Liu, B.H.Chong, S.N.Cao; J.Lumin., **142**, 155 **(2013)**.
[2] C.F.Bohren, D.R.Hufillann; Absorption and Scattering Light by Smal1 Particles, John Wiley & Sons, New York, **(1983)**.
[3] Y.Teng, F.Y.Ji, C.Li, Z.H.Yu, R.T.Liu; J.Lumin., **131**, 2661 **(2011)**.
[4] D.J.Li, T.Zhang, B.M.Ji; Spectrochim.Acta.A., **130**, 440 **(2014)**.
[5] A.Das, R.Thakur, A.Dagar, A.Chakraborty; Phys.Chem.Chem.Phys., **16**, 5368 **(2014)**.
[6] S.Krishnamoorthy, A.Shanmugam, I.Malaichamy; Dyes.Pigments., **112**, 210 **(2015)**.
[7] G.K.Wang, L.X.Wang, W.Tang, X.X.Hao, Y.L.Wang, Y.Lu; J.Fluoresc., **21**,1879 **(2011)**.
[8] F.Ding, G.Y.Zhao, J.L.Huang, Y.Sun, L.Zhang; Eur.J.Med.Chem., **44**, 4083 **(2009)**.
[9] M.Wagil, J.Maszkowska, A.Białk-Bielńska, M.Caban, P.Stepnowski, J.Kumirska; Chemosphere., **119**, S28 **(2015)**.
[10] L.L.Cheng, M.Wang, M.H.Wu, S.D.Yao, Z.Jiao, S.L.Wang; Spectrochim.Acta.A., **97**, 209 **(2012)**.
[11] P.L.V.Falé, L.Ascensão, M.L.M.Serralheiro, P.I.Haris; Spectrosc., **26**, 79 **(2011)**.
[12] J.Zhang, Q.S.Yan, J.P.Liu, X.H.Lu, Y.S.Zhu, J.Wang, S.J.Wang; J.Lumin., **134**, 747 **(2013)**.
[13] S.H.Li, D.J.Li; Spectrochim.Acta.A., **82**, 396 **(2011)**.
[14] S.Y.Bi, B.Pang, T.J.Wang, T.T.Zhao, W.Yu; Spectrochim.Acta.A., **120**, 456 **(2014)**.
[15] Z.J.Liu, X.F.Guo.Z.Feng, L.H.Jia; J.Solution.Chem., **44**, 293 **(2015)**.
[16] C.Z.Zheng, H.P.Wang, W.Xu, C.Y.Xu, J.G.Liang, H.Y.Han; Spectrochim.Acta A., **118**, 897 **(2014)**.
[17] F.Wang, W.Huang, B.Tang, X.J.Liu; Chin.J.Anal.Chem., **34**, S239 **(2006).**
[18] M.He, Z.N.Xia, Y.G.Yin, Z.Liu; Chin.J.Mod.Appl.Pharm., **21**, 429 **(2004).**
[19] Z.J.Liu,X.F.Guo, Z.Feng, L.H.Jia; J.Solution.Chem., **44**, 293 **(2015)**.
[20] Q.Wang, X.Y.Liu, M.Su, Z.H.Shi, H.W.Sun; Spectrochim.Acta.A., **136**, 321**(2015)**.
[21] B.K.Seth,A.Ray, S.Biswas, S.Basu; Metallomics, **6**, 1737 **(2014)**.
[22] S.Neamtu, N.Tosa, M.Bogdan; J.Pharm.Biomed.Anal., **85**, 277 **(2013)**.
[23] Y.Teng, F.Y.Ji, C.Li, Z.H.Yu, R.T.Liu; J.Lumin., **131**, 2661 **(2011)**.
[24] B.Bojko, A.Sulkowska, M.Maciazek-Jurczyk, J.Rownicka, W.W.Sułkowski; J.Pharm.Biomed. Anal., **52**, 384 **(2010)**.
[25] B.S.Liu, C.Yang, X.N.Yan, J.Wang, J.S.Zhang, Y.K.Lü; Spectrosc.Lett., **45**, 175 **(2012)**.