Study on Fluorescence Spectra of Thiamine, Riboflavin and Pyridoxine

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ABSTRACT

This paper presents the intrinsic fluorescence characteristics of vitamin B1, B2 and B6 measured with 3D fluorescence Spectrophotometer. Three strong fluorescence areas of vitamin B2 locate at λex/λem=270/525nm, 370/525nm and 450/525nm, one fluorescence areas of vitamin B1 locates at λex/λem=370/460nm, two fluorescence areas of vitamin B6 locate at λex/λem=250/370nm and 325/370nm were found. The influence of pH of solution to the fluorescence profile was also discussed. Using the PARAFAC algorithm, 10 vitamin B1, B2 and B6 mixed solutions were successfully decomposed, and the emission profiles, excitation profiles, central wavelengths and the concentration of the three components were retrieved precisely through about 5 iteration times.

Keywords: Thiamine/Vitamin B1, Riboflavin/Vitamin B2, Pyridoxine/Vitamin B6, Fluorescence Spectra, PARAFAC

1. INTRODUCTION

B vitamins are water-soluble and of great importance for the health of people, they can not be synthesized within the body, but be obtained from daily food, so the content measurement of B vitamins in the food is necessary for food security and research. Riboflavin, known as vitamin B2, is an easily absorbed micronutrient with a key role in maintaining health in humans and animals. As such, vitamin B2 is required for a wide variety of cellular processes. Vitamin B2 plays a key role in energy metabolism, and is required for the metabolism of fats, ketone bodies, carbohydrates, and proteins [1]. Thiamine, known as vitamin B1, is used in many different body functions and deficiencies may have far reaching effects on the body, yet very little of this vitamin is stored in the body, and depletion of this vitamin can happen within 14 days. Vitamin B6 can be found in a variety of forms in the foods we eat as well as in our bodies, it is involved at several steps in the metabolism of carbohydrates, is one of several B vitamins required for proper production of messaging molecules in our nervous system and brain. Vitamin B6 has been proven to play a role in the development of healthy immune system function and plays a well-researched role in the synthesis and metabolism of certain nervous system messaging molecules.

PARAFAC, a three way-decomposition method, has been found to be very useful in identifying the independent spectra of different types of fluorophores [2]. Compared to its predecessor, Principal Component Analysis (PCA) technique, PARAFAC provides both a quantitative and qualitative model of the data and separates the complex signal measured into its individual underlying fluorescent phenomena with specific excitation and emission spectra. It can track even small variations in EEM datasets by separating several independent groups of fluorophores from the overlapped components with a high resolution, so it is commonly used technique to monitor the mixed fluorescence EEMs. On the

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Seventh International Symposium on Precision Mechanical Measurements, edited by Liandong Yu, Proc. of SPIE Vol. 9903, 99030H · © 2016 SPIE
CCC code: 0277-786X/16/$18 · doi: 10.1117/12.2211248

other hand, the weakness of PARAFAC model may include the assumption of the independence among the estimated components in the model, and potential inclusion of one or more poorly estimated components, which may significantly affect the spectra and scores of all other components [3].

Ni reported that PARAFAC was applied to resolve the mixed and overlapped spectra of vitamin B1, B2 and B6 [4], and Agranovski [5] studied the NADH, NADPH and riboflavin with UVAPS and other instruments. Accordingly, in this paper, discussed not only the fluorescence spectra of riboflavin and thiamine individually, but also the overlap of fluorescence spectra of vitamin B1, vitamin B2 and vitamin B6 within the Ex/Em area with wavelength of 200~550/370~610nm, and by means of Parallel factor analysis (PARAFAC) method.

2. EXPERIMENTAL SECTION

2.1 Instruments and Reagents

The Molecular ΣH_2O ultra pure water machine (Shanghai Molecular Co. Ltd) was used to generate the ultra purified water, UPW whose pH value is 5.4. The vitamin B2 mother liquid were compounded with Riboflavin from Amresco co.Ltd whose purity greater than or equal to 98%, the vitamin B1 from Sigma co.Ltd with purity greater than or equal to 99%, and the vitamin B6 from Sigma co.Ltd with purity greater than or equal to 98%. All reagents and materials were weighed with Mettler Toledo precise electronic balance, and dissolved with Briton Robson Buffer with different pH values (1.95, 5.4, 5.66, 8.0 and 11.92).

The solvents used in the experiments include 0.2mol/L Na_2HPO_4 buffer, 0.1mol/L $C_6H_8O_7 \cdot H_2O$ buffer, Na_2HPO_4 and $C_6H_8O_7 \cdot H_2O$ mixed buffer, 1% $K_3[Fe(CN)_6]$ buffer, 2mol/L NaOH buffer and 3mol/L H_2SO_4 buffer. 0.2mol/L Na_2HPO_4 buffer was compounded by putting 28.4g sodium phosphte dibasic anhydrous (Na_2HPO_4, Tianjin guangfu chemical research institute, China) into 1000mL UPW. 0.1mol/L $C_6H_8O_7 \cdot H_2O$ buffer was compounded by put 21.01g citric acid monohydrate($C_6H_8O_7 \cdot H_2O$, Sinopharm Chemical Reagent Co.Ltd) into 1000mL UPW. Na_2HPO_4 and $C_6H_8O_7 \cdot H_2O$ mixed buffer was compounded by mixing 0.2mol/L Na_2HPO_4 buffer and $C_6H_8O_7 \cdot H_2O$ buffer at the ration of 6:1; 1% $K_3[Fe(CN)_6]$ buffer was compounded by put 1g Potassium hexacyanoferrate buffer($K_3[Fe(CN)_6]$, Tianjin guangfu chemical research institute) into 100mL UPW, and was diluted by mixing with 3mL 2mol/L NaOH buffer to 10mL.

The vitamin B2 and B1 mother liquid concentration are 10mg/L respectively. The vitamin B2 and vitamin B1 mother reagent solutions were transferred through DragonLab whole disinfection manual single channel adjustable liquid shifter and dilute to working solutions of d ifferent concentrations. All reagents were of analytical grade, all solutions and put in amber glass bottles and stored in a refrigerater (4°C) because of the light sensitivity of vitamin B2.

3D fluorescence intensity measurements were carried out on an F-7000 FL spectrophotometer (Hitachi High-Technologies Corporation, Japan).

2.2 Instrument Settings and Experiment Procedure

500ul Briton Robson Buffers with different pH values and mother liquids of different volumes were injected into the 10ml test tubes, and diluted with purified water to form the working liquids and background liquids.

For the fluorescence EEM measurements of vitamin B2, the spectrophotometer excitation wavelength ranged from 200.0nm, to 550.0nm, emission wavelength ranged from 450.0nm to 650.0nm, scan speed was set at 12000nm/min with excitation and emission sampling interval of 10.0 nm, excitation and emission slit of 5.0nm, the PMT voltage was set at

700 V. Accordingly, for fluorescence EEM of thiamine, the excitation wavelength ranged from 300.0nm, to 400.0nm, emission wavelength ranged from 350.0nm to 550.0nm. All experiments were performed at room temperature at 25°C

The 1st level and 2nd level Rayleigh scattering, Raman scattering and other background components within the fluorescence signals were corrected for the following analysis.

2.3 PARAFAC Method for Multi-components Discrimination

Based on the tri-linear decomposition theory, the parallel factor analysis(PARAFAC) method is a kind of mathematical model implemented through alternating least squares algorithm, which is widely applied to analyze three-dimensional or multi-dimensional data, to decompose *N*-dimensional data to the N load matrixes.

The measured fluorescence spectrum EEM data is a $I \times J \times K$ matrix, in which, *I* indicates the number of the samples, while *J* and *K* are the number of excitation wavelengths and emission wavelengths of samples respectively. Using parallel factor decomposition model, the fluorescence spectrum data matrix can be decomposed to score matrix *A*, load matrix *B* and *C*. The decomposition model can be represented as

$$x_{ijk} = \sum_{f=1}^{F} \alpha_{if} b_{jf} c_{kf} + \varepsilon_{ijk}, \; i=1, 2, \ldots, I, \quad j=1, 2, \ldots, J, \; k=1, 2, \ldots, K \tag{1}$$

where, x_{ijk} is the fluorescence intensity of sample *i* at excitation wavelength *j* and emission wavelength *k*, *F* is the column number of load matrix, or the number of factors, ε_{ijk} is the residual element, $\alpha_{if}, b_{jf}, c_{kf}$ are the elements in load matrix *A*, *B* and *C* respectively. The algorithm will be aborted until convergence of the PARAFAC model, that is, the minimum loss function

$$f_{SSR} = \sum_{i=1}^{I} \sum_{j=1}^{J} \sum_{k=1}^{K} e^2{}_{ijk} < 10^{-6} \tag{2}$$

In this study, PARAFAC modeling was performed using the MATLAB 7.0 code. The appropriate number of components was determined primarily based on the three diagnostic tools including residual analysis, core consistency and visual inspection of spectral shapes of each component, which are widely used by other similar studies. The components extracted by PARAFAC represent groups of the organic components that exhibit similar fluorescence properties. The component scores indicate the relative concentration of the groups, not the actual concentration of a particular material/fluorophore. However, it is typically assumed that the scores are proportional to the concentrations of the different components [6, 7].

3. FLUORESCENCE EEM CHARACTERISTICS OF THREE KINDS OF VITAMIN B

3.1 Intrinsic Fluorescence EEM Characteristics of Riboflavin, Thiamine and Pyridoxine

For riboflavin/vitamin B2, there are three strong fluorescence areas, whose center locate at λex/λem=270/525nm, 370/525nm and 450/525nm respectively, and the emission wavelength ranges from about 500nm to 600nm, as shown in

Figure 1(a). The fluorescence intensity excited by 270nm excitation wavelength is much stronger than that by 370nm and 450nm, the ratio of fluorescence intensity is 1:0.41:0.25 approximately.

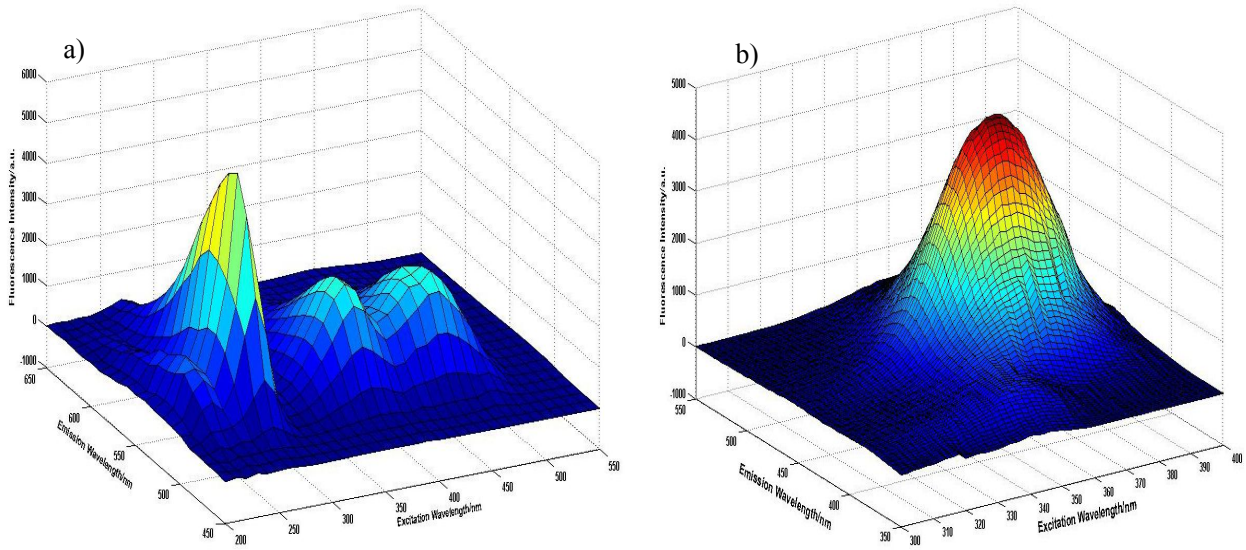


Figure1. Fluorescence intensity distribution of riboflavin@200ug/L and thiamine@75ug/L

And for thiamine/vitamin B1, it has only one strong fluorescence areas, whose center locate at λex/λem=370/460nm, the excitation wavelength ranges from about 320nm to 400nm, and the emission wavelength ranges from about 370nm to 550nm, as shown in Figure 1(b).

Accordingly, as for pyridoxine/bitamin B6, there are two strong fluorescence areas, whose center locate at λex/λem=240/370nm and 320/370nm respectively, and the emission wavelength ranges from about 330nm to 440nm, as shown in Figure 3. The fluorescence intensities excited by both excitation wavelengths are almost at the same altitude.

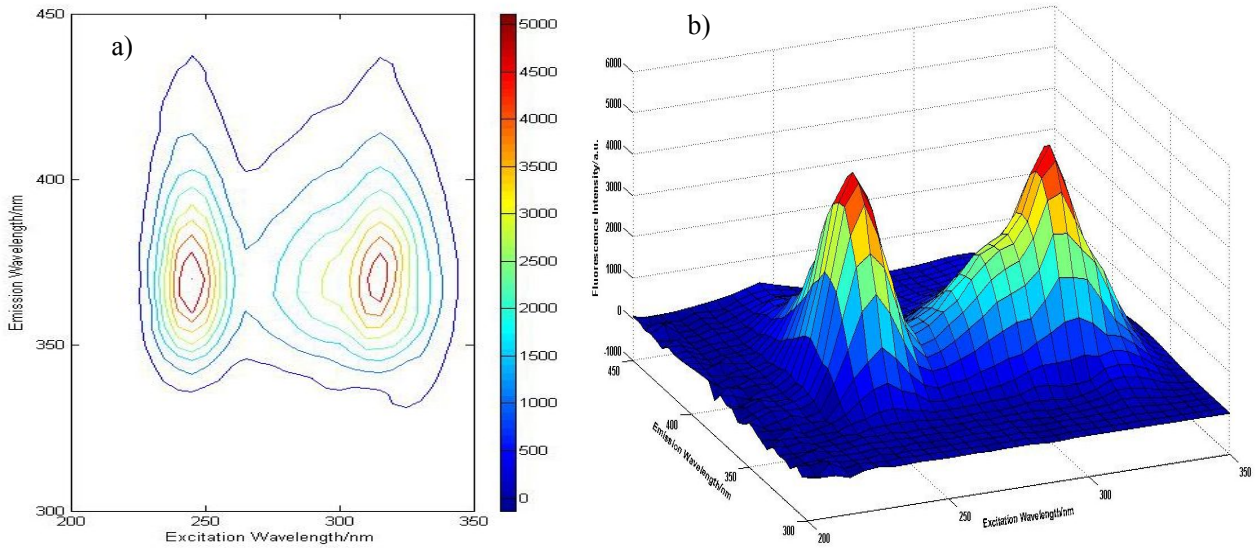


Figure2. Fluorescence intensity distribution of pyridoxine@200ug/L

3.2 Affection of pH to the fluorescence emission intensity distribution of riboflavin

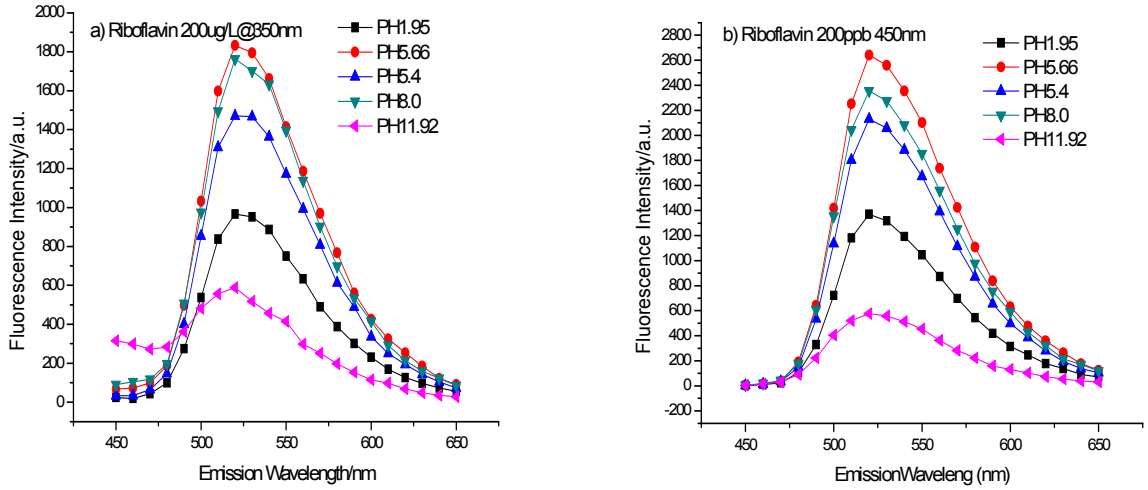


Figure3. Fluorescence intensity distribution of riboflavin@350nm and 450nm affected by pH value of the resolvent.

When resolved in strong alkaline and strong acid solution, the structure of vb2 molecular is distorted, so the fluorescence intensity declines sharply compared to the weak alkaline and weak acid solution, and if resolved in weak alkaline and weak acid solution, the fluorescence intensity are much stronger(figure3).

4. ANALYSIS OF FLUORESCENCE SPECTRA OF THREE COMPONENTS MIXED SOLUTIONS

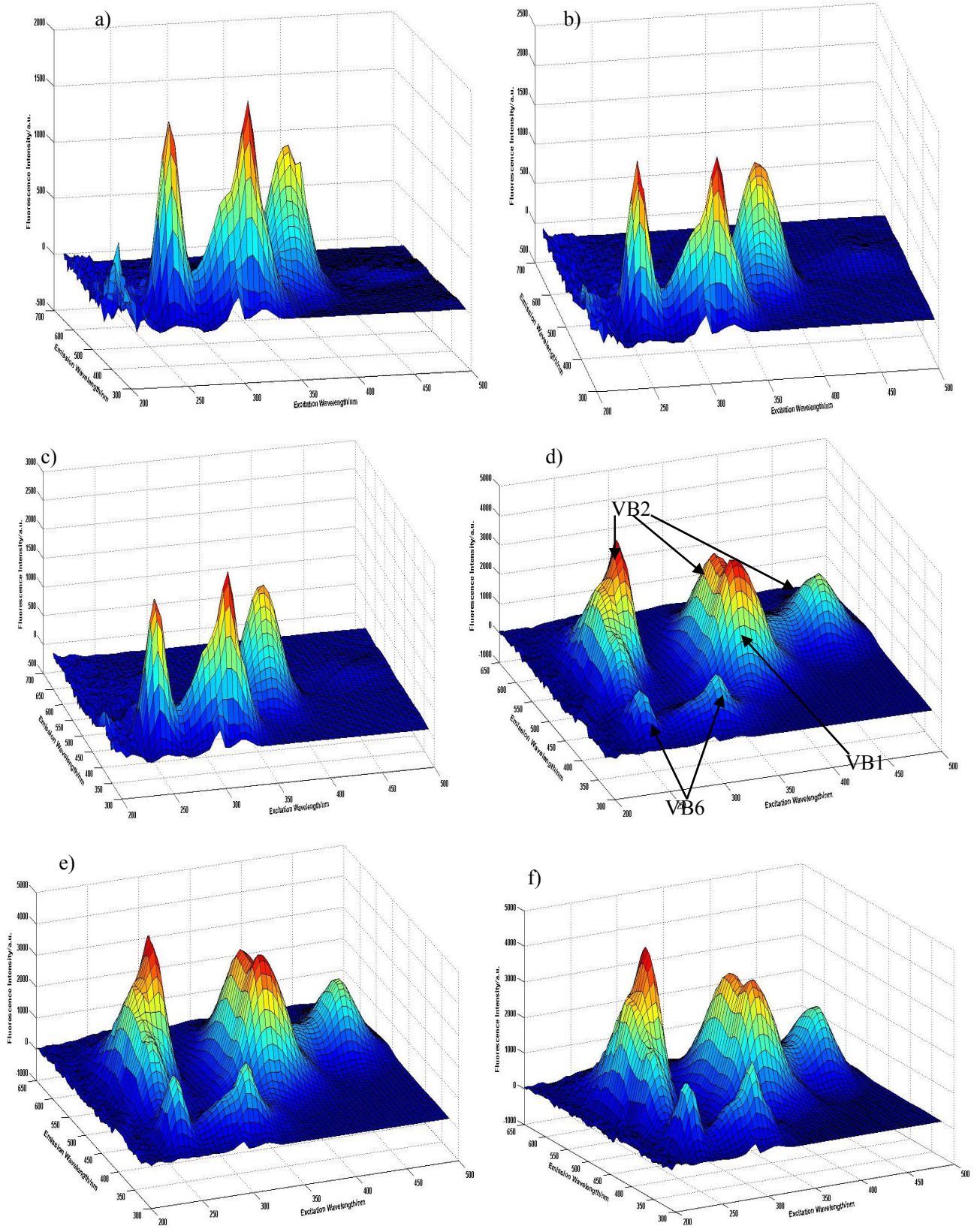
4.1 Instrument Settings

Firstly thiamine, riboflavin and pyridoxine mother liquids of different concentrations were put into 10mL numbered test tubes, and then 1mL Na2HPO4 and C6H8O7·H2O mixed buffer and 0.22ml K3[Fe(CN)6] buffer were added into the test tubes. The pHs of mixed solutions were then adjusted to neutral with added 3mol/L H2SO4 and UPW. Lastly, the mixed solutions were laid stationary for 10 minutes for the following measurements.

For the fluorescence EEM Matrixes decompose of the mixed solution of thiamine, riboflavin and pyridoxine, the spectrophotometer excitation wavelength ranged from 200.0nm, to 500.0nm, emission wavelength ranged from 330.0nm to 650.0nm, scan speed was set at 12000nm/min with excitation and emission sampling interval of 5.0 nm, excitation and emission slit of 10.0nm, the PMT voltage was set at 700 V.

4.2 3D Fluorescence Spectrogram of Three Components Mixed Solutions

Fluorescence EEM intensity of three components mixed solutions of different concentrations (listed in table1) are shown in figure4 (a) ~ (j). from figure4 (d) ~ (j), the separated fluorescence EEM peaks of vitamin B2 at 260nm/525nm and 470nm/520nm are obviously, the merged fluorescence EEM peak of vitamin B2 and B1 at excitation wavelength 370nm, and the merged fluorescence EEM peak of vitamin B2, B1 and B6 centered at 340nm/360nm are also distinctive.



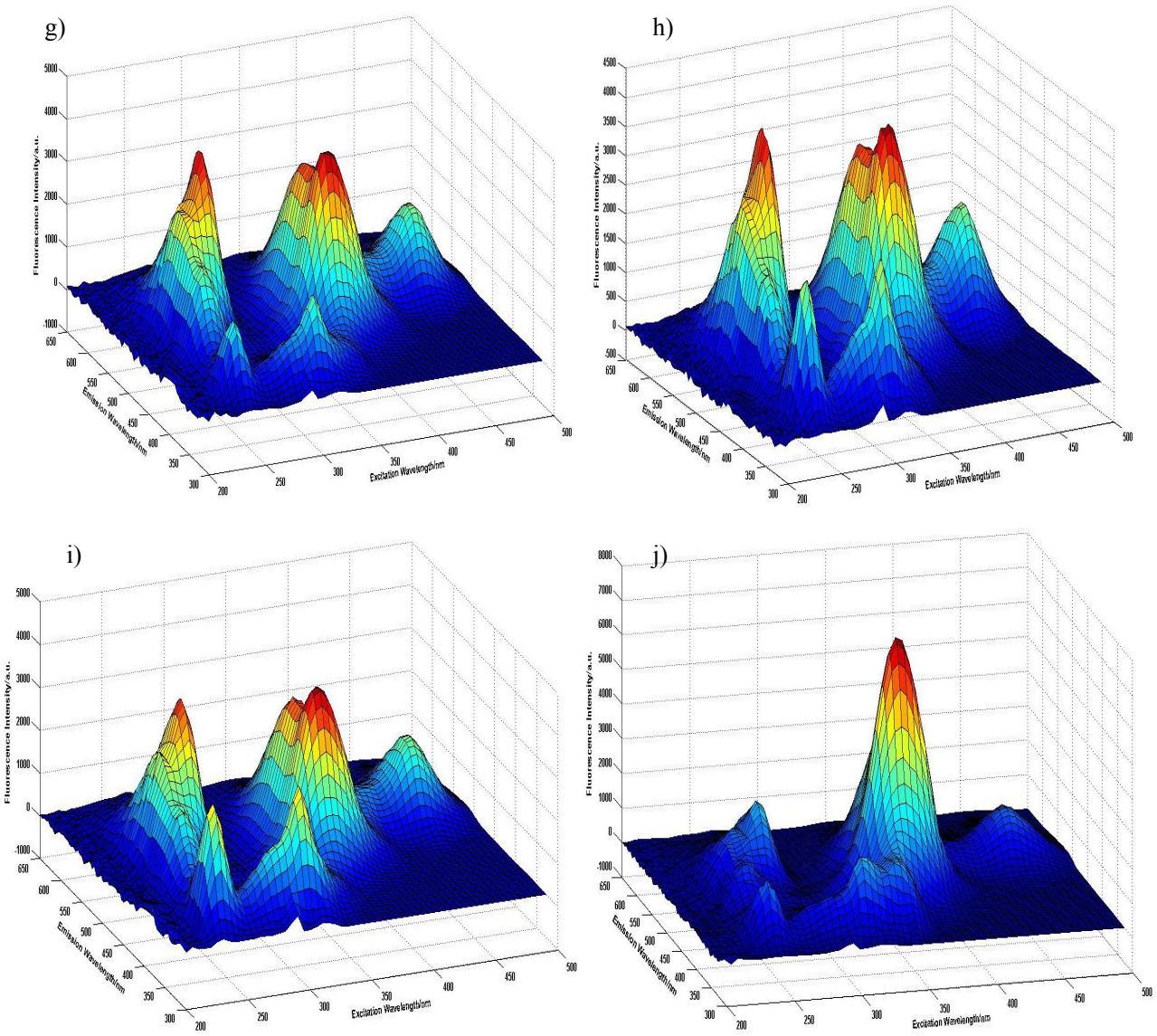


Figure4. Fluorescence EEM intensity distribution of riboflavin and thiamine mixed solutions of different concentrations. a) vb1 10ug/mL, vb2 10ug/mL, vb6 100ug/mL; b) vb1 20ug/mL, vb2 20ug/mL, vb6 150ug/mL; c) vb1 20ug/mL, vb2 40ug/mL, vb6 200ug/mL; d) vb1 25ug/mL, vb2 100ug/mL, vb6 100ug/mL; e) vb1 25ug/mL, vb2 150ug/mL, vb6 150ug/mL; f) vb1 25ug/mL, vb2 200ug/mL, vb6 200ug/mL; g) vb1 50ug/mL, vb2 100ug/mL, vb6 100ug/mL; h) vb1 50ug/mL, vb2 150ug/mL, vb6 150ug/mL; i) vb1 50ug/mL, vb2 200ug/mL, vb6 200ug/mL; j) vb1 100ug/mL, vb2 100ug/mL, vb6 100ug/mL.

4.3 Results Retrieved by PARAFAC Algorithm

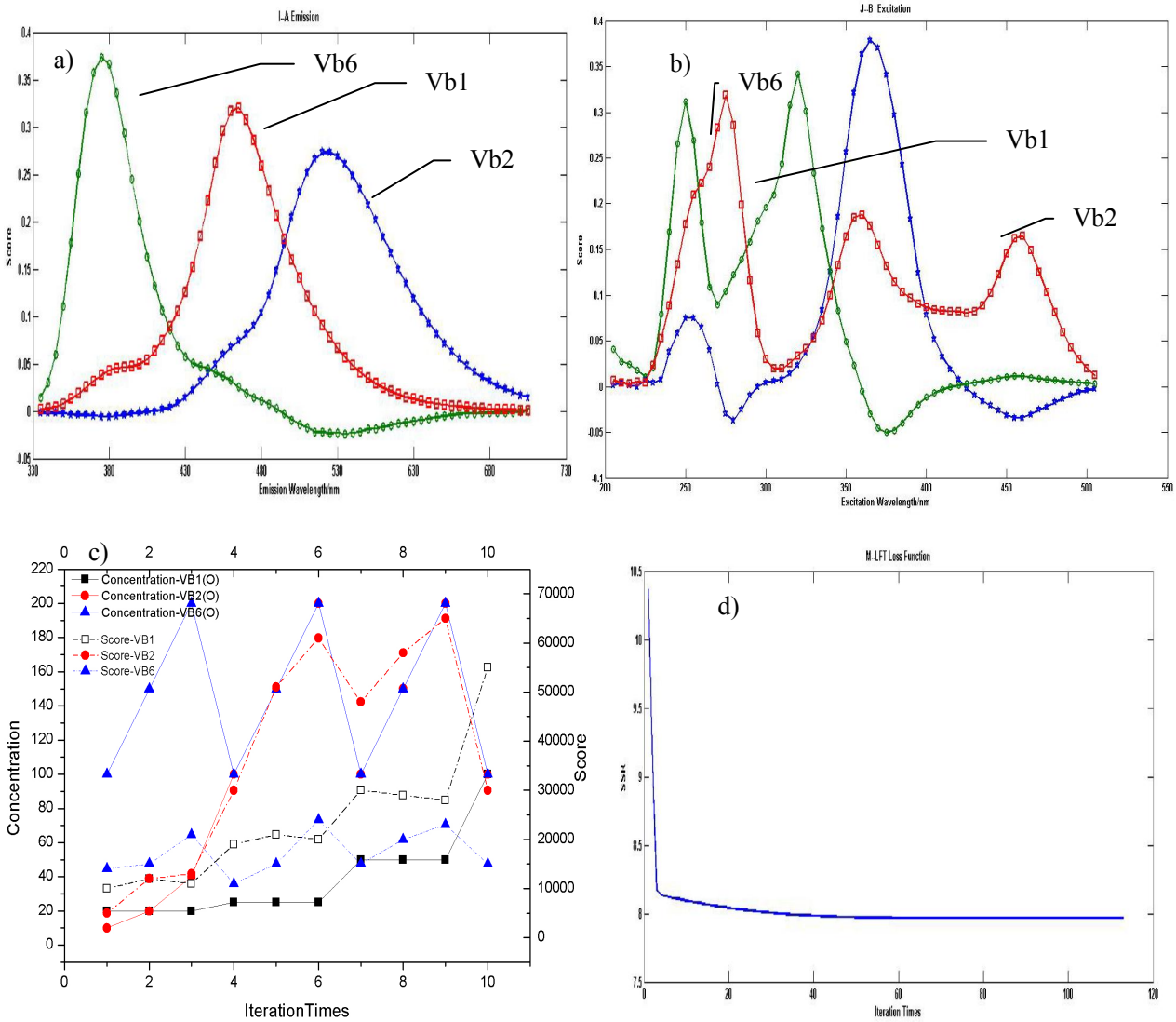


Figure5. Excitation and emission spectral profile retrieved by PARAFAC algorithm, (a)Emission spectra, (b) Excitation spectra, (c) original and retrieved concentrations, (d) SSR and iteration number

The emission spectra retrieved by PARAFAC are shown in Figure 5(a), the green curve with circle symbol indicates the retrieved emission profile of pyridoxine, the red curve with block symbol indicates the retrieved emission profile of thiamine and the blue curve with star symbol indicates the retrieved emission profile of riboflavin. From figure 5(a) and figure 1 to 2, one can see that the retrieved emission profile and central wavelength of riboflavin, thiamine and pyridoxine are coincident with their real emission profiles very well.

The excitation spectra retrieved by PARAFAC are show in Figure 5(b), the green curve with circle symbol indicates the excitation profile of pyridoxine, the red curve with block symbol indicates the excitation profile of riboflavin, and the blue curve with star symbol indicates the excitation profile of thiamine. From figure 5(b) and figure 1, figure 2, it can be seen that the retrieved excitation profile and central wavelength of riboflavin and pyridoxine are coincident with their

real excitation profiles. But as for the retrieved excitation profile and central wavelength of pyridoxine, there exists a extra weak excitation peak at 250nm.

The good linear correlations of original and retrieved concentrations of thiamine, riboflavin and pyridoxine can be observed in Figure 5(c). From Figure 5(d) one can see that SSR of PARAFAC logarithms decreases quickly and sharply at the beginning of the iteration times, the SSR is stable when iteration times≥5.

Table 1. Analytical concentrations of riboflavin and thiamine

Sample	Component					
	Thiamine		Riboflavin		Pyridoxine	
	Original (ug/L)	Retrieved score	Original (ug/L)	Retrieved score	Original (ug/L)	Retrieved score
a)	20	10000	10	1000	100	14000
b)	20	12000	20	1200	150	15000
c)	20	15000	40	1300	200	21000
d)	25	29000	100	50000	100	11000
e)	25	31000	150	53000	150	15000
f)	25	25000	200	51000	200	14000
g)	50	30000	100	48000	100	15000
h)	50	29000	150	47000	150	20000
i)	50	28000	200	45000	200	23000
j)	100	55000	100	30000	100	15000

5. CONCLUSIONS

The fluorescence of a protein or bio-aerosol or bio-agent is a mixture of the fluorescence from individual aromatic residues and coenzyme. Using fluorescence Spectrophotometer, the intrinsic fluorescent characteristics of vitamin B1, B2 and B6 were measured with solutions of different concentration and the effect of pH to fluorescent profile was also discussed. Vitamin B1, B2 and B6 mixed solutions are successfully decomposed and resolved by PARAFAC algorithm. The retrieved emission profiles, excitation profiles, central wavelengths and the concentration of three components are coincident precisely with real emission profiles, excitation profiles, and central wavelengths of each component.

ACKNOWLEDGMENTS

The work was supported by the National Natural Science Foundation of China named “Study on Technology of Ultraviolet Laser-induced Fluorescence LIDAR for Bioagent Remote Sensing” No. 41375026.

REFERENCES

[1] National Academy of Sciences, 2004. “Sensor Systems for Biological Agent Attacks: Protecting Buildings and Military Bases, Committee on Materials and Manufacturing Processes for Advanced Sensors, Board on Manufacturing and Engineering Design, Division on Engineering and Physical Sciences”, National Academies Press Washington URL: http://www.national-academies.edu/bmed

[2] Stedmon, C.A.; Markager, S.; Bro, R. “Tracing dissolved organic matter in aquatic environments using a new approach to fluorescence spectroscopy”. Mar. Chem. 82, 239-254(2003).

[3] Murphy, K.R.; Hambly, A.; Singh, S.; Henderson, R.K.; Baker, A.; Stuetz, R.; Khan, S.J. “Organic matter fluorescence in municipal water recycling schemes: Toward a unified PARAFAC model”. Environ. Sci. Technol., 45, 2909-2916(2011).

[4] Ni Yong-nian, Cai Ying-jun, “Simultaneous synchronous spectrofluorimetric determination of vitamin B1,B2 and B6 by PARAFAC”, Spetroscopy and Spectral Analysis[J], 25(10): 1641-1644(2005)

[5] Victoria Agranovski, Zoran D. Ristovski, Godwin A. Ayoko, Lidia Morawsk, “Performance Evaluation of the UVAPS in Measuring Biological Aerosols: Fluorescence Spectra from NAD(P)H Coenzymes and Riboflavin”, Aerosol Science and Technology[J], 38:354-364(2004)

[6] Stedmon, C.A.; Bro, R. “Characterizing dissolved organic matter fluorescence with parallel factor analysis: A tutorial. Limnol”. Oceanogr. Methods, 6, 572-579(2008).

[7] Li Weihua.; Sheng, G.aoping.; Liu, Xianwei.; Yu H.anqing. “Characterizing the extracellular and intracellular fluorescent products of activated sludge in a sequencing batch reactor”. Water Res, 42, 3173-3181(2008)