Intrinsic Fluorescence Spectra of Tryptophan, Tyrosine and Phenyloalanine

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Abstract: This paper presents the intrinsic fluorescence spectra characteristics of tryptophan, tyrosine and phenyloalanine measured with 3D fluorescence spectrophotometer. Two strong fluorescence peaks of tryptophan locate at λex/λem=230/348nm and 280/348nm, three strong fluorescence peaks of tyrosine locate at λex/λem=202/304nm, λex/λem=220/304nm and 275/304nm, and two fluorescence peaks of phenyloalanine locate at λex/λem=210/280nm and λex/λem=260/280nm were found. The linear relationship of fluorescence intensity to solution concentration were also observed in condition of low solution concentration. The influence of pH of solution to the fluorescence intensity was also measured and discussed.

Introduction

Of the twenty naturally occurring amino acids that make all proteins, three are conjugated with aromatic ring side chains, and therefore intrinsically optically active: tryptophan (Trp), tyrosine (Tyr) and phenyloalanine (Phe). Tryptophan is excited at wavelengths around 280 nm and emits fluorescence in a peak from 300-400 nm. NADH is excited between 270 and 400 nm and emits between 400 and 600 nm, and Riboflavin is excited at 300-500 nm and emits mostly between 400 and 700 nm[1,2,3] . The fluorescence from a complex mixture of proteins mainly originates from Trp residues. The indole group of tryptophan residues is the dominant source of absorbance and emission in protein. And the excitation and emission bands of these bio-fluorophores are quite overlapped within the EEM area whose excitation wavelength/emission wavelength scope is 230-270nm/260-340nm. So through the detection of intrinsic fluorescence characteristics of tryptophan, tyrosine and phenyloalanine, the comparative contents of various kinds of organic components can be analyzed and resolved, and finally the classification of bio-aerosol/bio-agent can be reached.

In this paper, the intrinsic 3D fluorescence spectra of tryptophan, tyrosine and phenyloalanine were obtained with an F-7000 FL spectrophotometer and the individual excitation/emission spectra were decomposed successfully from the mixed solutions and simulated mixtures.

Experimental Section

Instruments and Reagents

The Molecular ΣH2O ultra pure water machine (Shanghai Molecular Co. Ltd) was used to generate the ultra purified water, UPW whose pH value is 5.4. L-Tryptophan mother liquid were compounded with L-Tryptophan from Sigma co.Ltd whose minimum purity equals to 99%, the L-Tyrosine mother liquid were compounded with L-Tyrosine from Sigma co.Ltd whose minimum purity equals to 98%, and the L-Phenylalanine mother liquid were compounded with L-

Phenylalanine from USB co.Ltd whose minimum purity equals to 99%. All reagents and materials were weighed with Mettler Toledo precise electronic balance, and dissolved with KH_2PO_4 buffer or Na_2HPO_4 buffer with different pH values of 6.5, 6.6, 6.8, 7.0, 7.2, 7.3, 7.4, 7.6 and 8.0.

The L-Tryptophan, L-Tyrosine and L-Phenylalanine mother liquid concentration are 20, 21 and 40 mg/L respectively. The all mother reagent solutions were transferred through DragonLab whole disinfection manual single channel adjustable liquid shifter and dilute to working solutions of different concentrations. All reagents were of analytical grade, all solutions and put in amber glass bottles and stored in a refrigerator (4˚C).

The solvents used in the experiments include 0.2mol/L Na_2HPO_4 buffer, 0.2mol/L NaH_2PO_4 buffer, 0.1mol/L NaOH buffer, 0.2mol/L NaOH buffer and KH_2PO_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. KH_2PO_4-NaOH mixed buffer of pH 7.4 was compounded by putting 1.36g potassium dihydrogen phosphate (KH_2PO_4, Tianjin fuchen chemical reagents factory, China) and 79ml NaOH buffer of 0.1mol/L into 200mL UPW.

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

Instrument Settings and Experiment Procedure

KH_2PO_4-NaOH Buffers, Na_2HPO_4 or NaH_2PO_4 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 L-Tryptophan, L-Tyrosine and L-Phenylalanine, the spectrophotometer excitation wavelength ranged from 200.0nm to 400.0nm, emission wavelength ranged from 210.0nm to 500.0nm. Scan speed was set at 12000nm/min with excitation and emission sampling interval of 2.0 nm, excitation and emission slit of 10.0nm, the PMT voltage was set at 600 V. All experiments were performed at room temperature at 25˚C.

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

Intrinsic Fluorescence EEM Characteristics of Tryptophan, Tyrosine and Phenyloalanine

For tryptophan whose fluorescence quantum yield is about 0.14, there are two strong fluorescence areas, whose center locate at λex/λem=230/350nm, 280/350nm respectively, and the emission wavelength ranges from about 270nm to 470nm, as shown in Figure 1. The fluorescence intensity excited by 280nm excitation wavelength is much stronger than that by 230nm excitation wavelength, and the ratio of fluorescence intensity is 1:1.10 approximately.

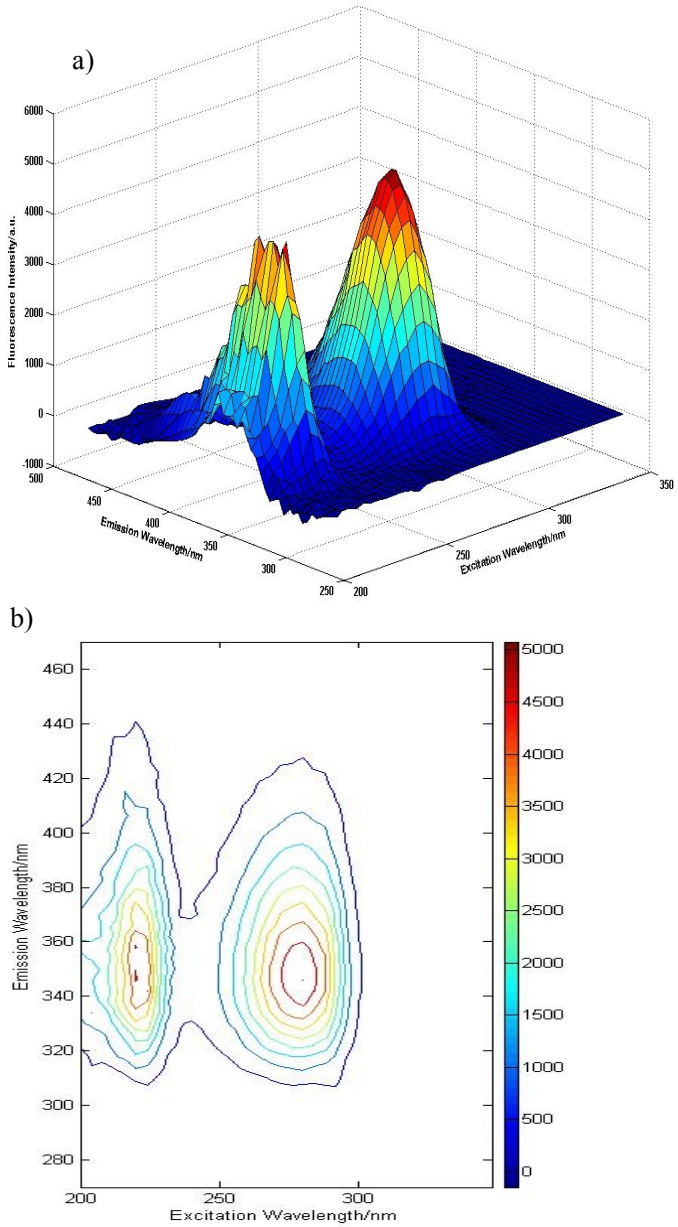


Figure1. Fluorescence intensity distribution of tryptophan@80ug/L

Tyrosine has three strong fluorescence areas, whose center locate at λex/λem=202/304nm, λex/λem=220/304nm and λex/λem=274/304nm, fluorescence intensity at the positions of λex/λem=202/304nm and λex/λem=220/304nm are stronger than that of its main peak at λex/λem=274/304nm, as shown in Figure 2.

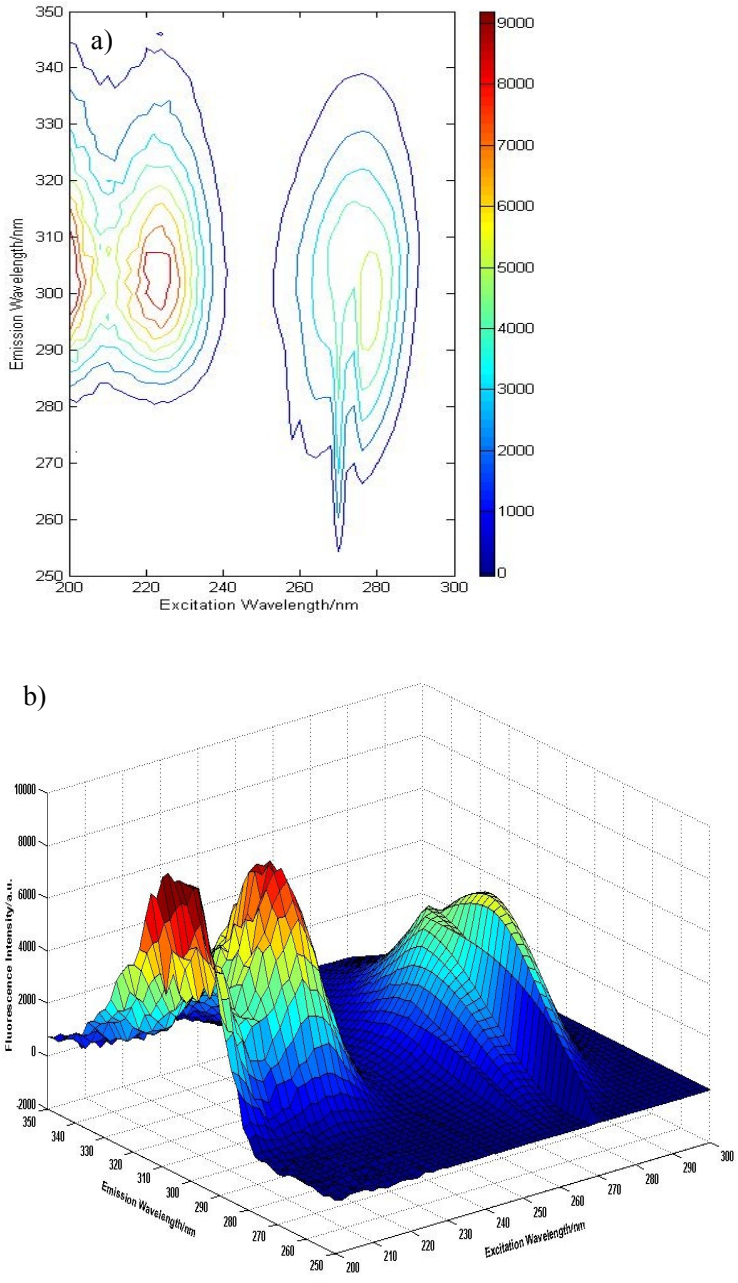
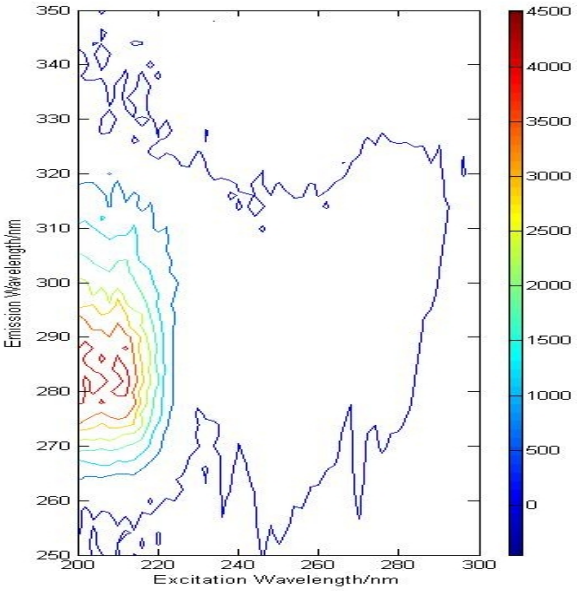


Figure2. Fluorescence intensity distribution of tyrosine@30ug/L

a)



b)

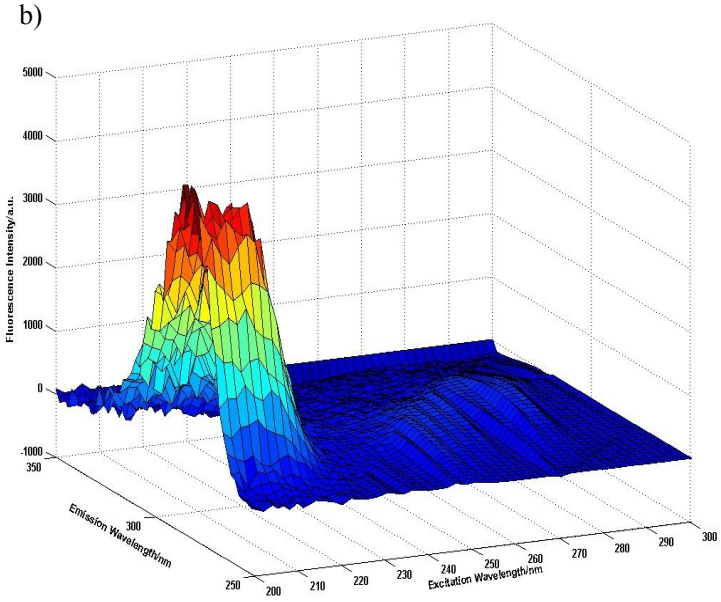
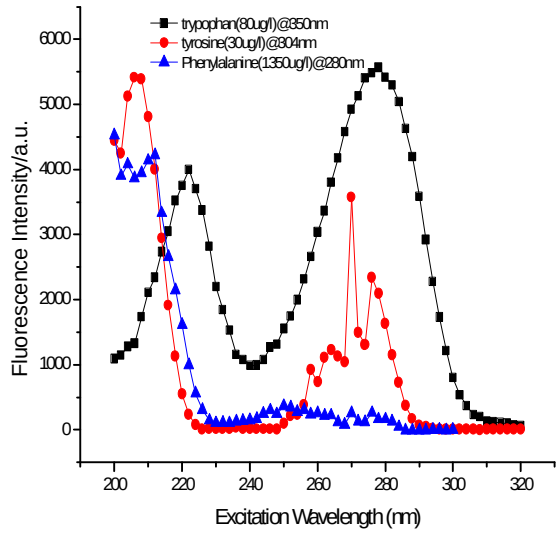


Figure3. Fluorescence intensity distribution of phenyloalanine @1350ug/L

And for phenyloalanine, there are two excitation wavelengths, the shorter one locates at 210nm and the longer one locates at 260nm. The fluorescence of the first peak at 210/280nm is much stronger than the second peak at 260/280nm, as shown in figure 3.

From figure 1 to 3, it can be conclude that, the excitation/emission wavelength of tryptophan, tyrosine and phenyloalanine locate at λex/λem=280/350nm, 275nm/303 and 260/280nm respectively, the overlapping of these three 3D fluorescence spectra is obvious, as shown in figure 4.



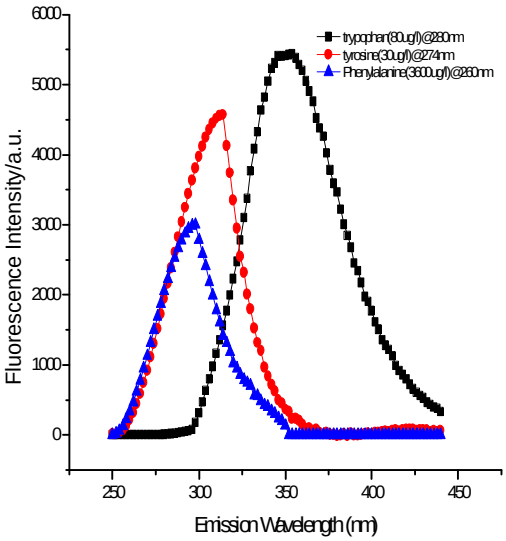
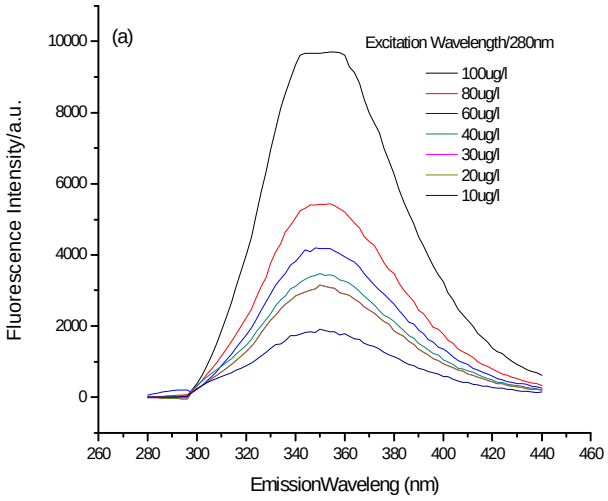
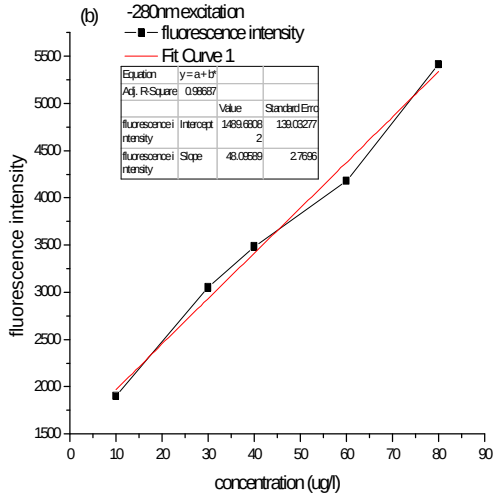


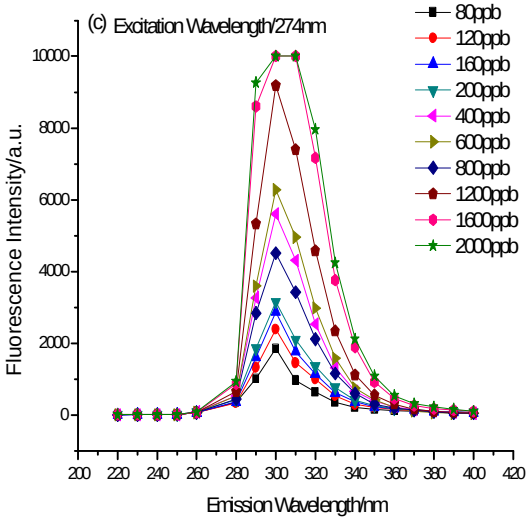
Figure4. Excitation and emission spectra of tryptophan, tyrosine and phenyloalanine @1350ug/L

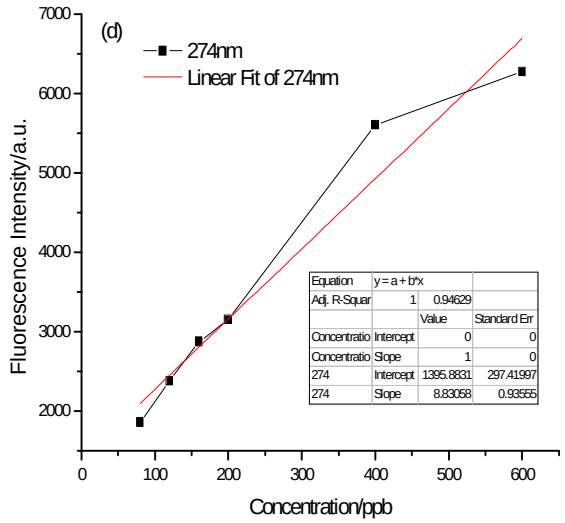
Linear Relationship of Fluorescence Intensity to Concentration

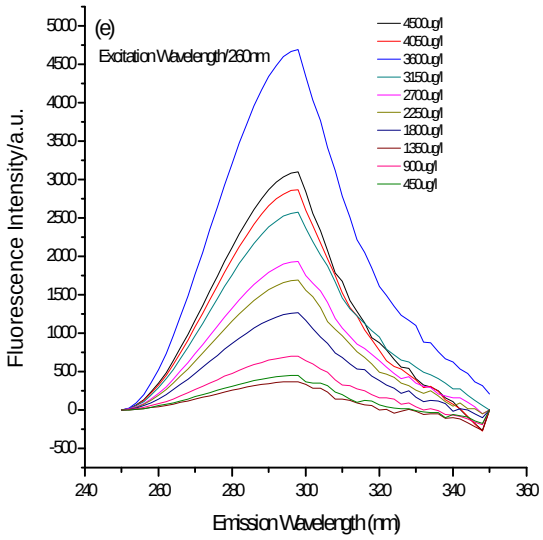
According to the Lambert-Beer law, when a monochromatic light beam passes through a homogeneous, non-scattering material, the optical energy will be absorbed much stronger with higher concentration of the resolution. So for the material capable of fluorescence emission, fluorescence intensity rises with higher concentration of the resolution, the fluorescence intensity is linear to the concentration of the material resolution. The emission spectra of tryptophan at λex=280nm and the linear relationship of fluorescence intensity to resolution concentration are shown in figure5(a) and (b), the emission spectra of tyrosine at λex=274nm and the linear relationship of fluorescence intensity to resolution concentration are shown in figure5(c) and (d), and the emission spectra of phenyloalanine at λex=260nm and the linear relationship of fluorescence intensity to resolution concentration are shown in figure5(e) and (f).


(a)
Excitation Wavelength/280nm
100ug/l
80ug/l
60ug/l
40ug/l
30ug/l
20ug/l
10ug/l
Fluorescence Intensity/a.u.
EmissionWaveleng (nm)


(b)
-280nm excitation
fluorescence intensity
Fit Curve 1
Equation y = a+ b*
Adj. R-Square 0.99887
Value Standard Erro
fluorescence intensity Intercept 1489.6808 139.03277
fluorescence intensity Slope 48.08589 2.7695
fluorescence intensity
concentration (ug/l)


(c) Excitation Wavelength/274nm
80ppb
120ppb
160ppb
200ppb
400ppb
600ppb
800ppb
1200ppb
1600ppb
2000ppb
Fluorescence Intensity/a.u.
Emission Wavelength/nm


(d)
274nm
Linear Fit of 274nm
Fluorescence Intensity/a.u.
Concentration/ppb
7000
6000
5000
4000
3000
2000
0
100
200
300
400
500
600
Equation y = a + b*x
Adj. R-Squar 1 0.94629
Value Standard Err
Concentratio Intercept 0 0
Concentratio Slope 1 0
274 Intercept 1395.8831 297.41997
274 Slope 8.83058 0.93555


(e)
Excitation Wavelength 260nm
4500ug/l
4050ug/l
3600ug/l
3150ug/l
2700ug/l
2250ug/l
1800ug/l
1350ug/l
900ug/l
450ug/l
Fluorescence Intensity/a.u.
Emission Wavelength (nm)
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-500
240
260
280
300
320
340
360

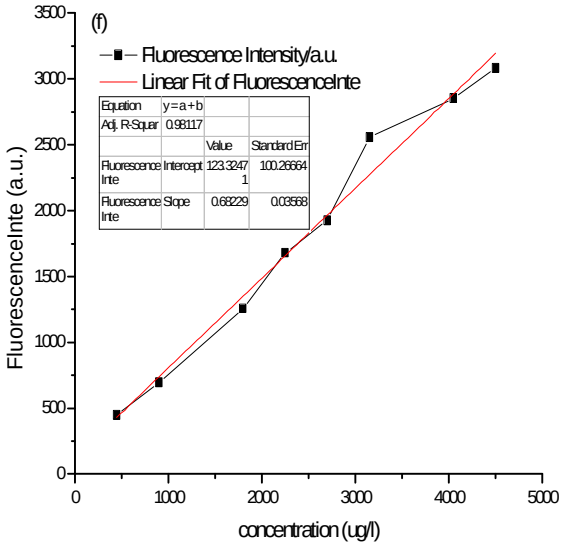


Figure5. Fluorescence spectra of tryptophan, tyrosine and phenyloalanine in different concentration at λex=280nm, λex=274nm and λex=260nm respectively

From figure 5, the linear regression equation were obtained. the linear correlation coefficient of tryptophan comes up to 0.98687, the fluorescence intensity is linear to the concentration of solution when the concentration is below 100ug/L. for phenyloalanine, the linear correlation coefficient is up to 0.98117 when the concentration is below 4500ug/L, and for tyrosine, the linear correlation coefficient declines to 0.94629 when the the concentration is below 600ug/L.

Affection of pH to the Fluorescence Emission Intensity

The pH affects the fluorescence emission intensities of the three components, especially the tryptophan and tyrosine. The fluorescence emission intensities of tryptophan and tyrosine will be strongest when the pH ranges from 6.5~7.5 and will decline sharply when pH is greater than 7.5, as shown in figure6.

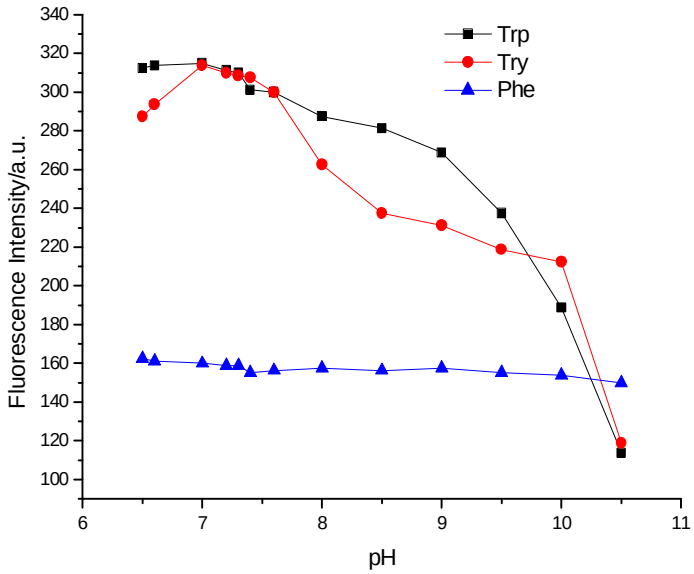


Figure6. Effect of pH/acidity of solution on fluorescence intensity of tryptophan, tyrosine and phenyloalanine respectively

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 tryptophan, tyrosine and phenyloalanine were measured with solutions of different concentration and the effect of pH to fluorescent profile was also discussed.

Acknowledgments

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