Effects of Varied Tyrosine Concentrations on FEEM and SFS of Constant Tryptophan concentration

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Abstract. In recent years, the 3D fluorescence excitation emission spectrum has been widely applied into the analysis of the properties of soluble organic matter in water bodies, but there is very little research on the fluorescence properties of protein residues in water quality detection by Fluorescence Excitation Emission Matrix (FEEM), and synchronous fluorescence excitation emission spectra (SFS). The characteristics of luminescent of amino acids, including tryptophan, tyrosine and phenylalanine, where tryptophan and tyrosine fluorescence are relatively strongest, and the effects of tyrosine on the fluorescence properties of tryptophan are seldom discussed in different mixing ratios for both amino acids. The fluorescent peak positions and intensities of the three amino acids are related to the angle size of conjugate plane, N hybrids and hydroxyls on benzene rings. When the ratio of tyrosine to tryptophan content is less than 150:100, tryptophan will quench the fluorescent peak of the tyrosine. Constant tryptophan content, tyrosine at excitation wavelength of 228 nm, its concentration changes with the corresponding fluorescence intensity has a good linear relationship; however, at 276-278 nm of tyrosine, the varied concentrations and fluorescence intensities are opposite.

1. Introduction

The soluble organic matter, 30-40% of total organic pollutants in city sewage [1], is the major target in sewage treatment. Monitoring the amount and characteristic of organic matter is always an important item in research of sewage treatment. Conventional organic pollutant parameters like chemical oxygen demands (COD), total organic carbons (TOC) can be only used to indicate the amount of organic matter but not the characteristics and ingredients of organic matter, which also need more water sample volume and operating time. In recent years, highly sensitive and using little sample volume, the 3D fluorescence excitation emission spectrum has been widely applied into the analysis of the properties of soluble organic matter in water bodies [2,3]. It reported that as proteins, humus and grease in life sewage possess fluorescence, 3D fluorescence excitation emission spectrum can be developed as a new method of indicating organic matter [4,5]. Chen et al divided the 3D fluorescence excitation emission spectrum of city sewage into five fingerprint areas. Otherwise, the area for protein amino acid residues was seldom researched in more detail, especially for the first and second area belonging to themselves and the fourth area belonging to solute products of microbial degradation [9,10].

Currently, most studies on the fluorescence properties of amino acids in water are those of molecular structure and fluorescence properties, the effects of fluorescent additives on the fluorescence properties...
of amino acids, correlation between fluorescence properties and COD, the difference of the fluorescence properties of amino acid residue from different sources. However, the fluorescence spectrum of single amino acid solution has been seldom studied, especially for that of amino acid mixture, and the mutual effects of coexisting of two type of amino acid residues on fluorescence properties have been little reported [11,12].

Therefore, this paper will get on the research of 3D fluorescence excitation emission spectrum for the artificial solutions of tryptophan, tyrosine and phenylalanine, and the mixtures of tryptophan and tyrosine will be detected to discuss the mutual effects of EEFM and SFS.

2. Materials and Methods

2.1. Preparation of reagents and samples
Tree kinds of fluorescent samples: tryptophan, tyrosine and phenylalanine with a purity of more than 99% were bought from Sigma company. 1000 mg/L each sample solution was prepared by weighting the sample accurately to 0.0001 g and using free-ion water to dissolved it completely. Other 5 mg/L, 0.1 mg/L sample solutions were prepared by dilution from 1000 mg/L.

2.2. Instrument and experimental condition
Fluorescence spectrometer (F-4500, Hitachi, Japan) equipped with a 150 w xenon light and a 700 V photomultiplier tube detector was used to scan samples at a single wavelength or also at full wavelength to obtain their 3D spectrum

2.2.1 Fluorescent Excitation Emission Matrix(FEEM)
Full-spectrum scanning was completed under the following operating conditions: excitation walength:200-400 nm, emission wavelength: 250-550 nm, grating length: 10 nm, scan spacing: 2 ,3 nm respectively, scan velocity of 2400 nm/min.

2.2.2 Simultaneous Fluorescent Scanning(SFS)
Simultaneous fluorescent scanning was completed under the following operating conditions: excitation walength:200-550 nm, emission wavelength: 270 nm, grating length: 10 nm, scan velocity: 240nm/min.

3. Results and discussions

3.1 Characteristic analysis of 3D fluorescence excitation emission spectrum
50 mg/L tryptophan, tyrosine and phenylalanine solutions were scanned individually by 3D fluorescence spectrometer and the fluorescent intensities all exceeded the upper limit of this instrument. Therefore, the lower concentration (0.1 mg/L) diluted solutions were detected to obtain their spectrum (Figure 1). The results of comparison for target peak intensity of three amino acids indicate that tyrosine is higher than tryptophan, and phenylalanine is the lowest. In order to view the spectrum of phenylalanine more clearly, 5 mg/L phenylalanine solution was scanned to obtain Figure 1 C-2. The positions of two target peaks for their spectrums were Ex/Em: 226/301nm(1270) and 214/304 nm(1212) for 0.1 mg/L tyrosine solution, Ex/Em: 222/349 nm (1328) and 278/352 nm (2585) for 0.1 mg/L tryptophan solution, and Ex/Em: 214/280 nm (1502) and 258/283 nm (1225) for 0.5 mg/L phenylalanine solution. The two target peaks for every one of three amino acids can be attributed to two conjugated plane structures in their molecular structure including an aromatic ring and a carboxyl acid group. which are not coplanar each other [15]. Because of the more rigid structure of benzene ring adjacent with N-hetercyclo ring for Tryptophan, the fluorescent intensity is more than the other two and the peak emission wavelength is red shift. Comparing with Phenylalanine, the fluorescent intensity and wavelength of Tyrosine is higher than Phenylalanine due to the phenol group which makes the conjugated length longer than benzene ring.
Figure 1. FEEM of (A) 0.1 mg/L Tyrosine (B) 0.1 mg/L Tryptophan (C-1) 0.1 mg/L Phenylalanine (C-2) 5 mg/L Phenylalanine

Figure 2 indicates the simultaneous fluorescent spectrum ($\Delta \lambda = 70$ nm) of 0.1 mg/L Tyrosine, Tryptophan, Phenylalanine and 0.5 mg/L Phenylalanine. Two peaks of spectrum for tyrosine are found at excitation wavelength 210-250 nm and 250-300 nm respectively.

Figure 2. SFS diagram of tyrosine, tryptophan, and phenylalanine ($\Delta \lambda = 70$ nm)

The intensity of the peak at 210-250 nm wavelength are much more than that at 250-300 nm wavelength. The single peak of spectrum for tryptophan happens at 250-300 nm wavelength, which may be a combined peak from two peaks at near position. Because the intensity of the peak of spectrum of 0.1 mg/L Phenylalanine is too weak, we explore the spectrum of 0.5 mg/L Phenylalanine. Two peaks of
spectrum for Phenylalanine are found at excitation wavelength 200-230 nm and 230 nm respectively. Chu et al. had reported that the excited wavelengths for the spectra of Tryptophan, Tyrosine and Phenylalanine were selected at 279 nm, 224 nm and 256 nm, respectively, which meets the results of our study [16].

3.2 Characteristic analysis of simultaneous fluorescent spectrum of Tyrosine and Tryptophan
Molecular structures of the proteins in water generally have the residue of Tyrosine or Tryptophan. Therefore, the fluorescent spectra of mixtures of Tyrosine and Tryptophan were explored to understand the change of spectrum before and after mixing them. Figure 3.

Figure 3. FEEMs of constant concentration of tryptophan (100 µg/L) with varied tyrosine concentrations of (A)50 µg/L(B) 100 µg/L(C)150 µg/L(D) 200 µg/L (E) 300 µg/L (F) 400 µg/L

Was the spectra of mixtures for 100 µg/L Tryptophan solution, which is constant in total concentration, with various concentration: 50, 100, 150, 200,300 and 400 µg/L tyrosine solutions. Figure3A indicates the peaks of the spectrum of the mixture for 100 µg/L Tryptophan with 50 µg/L Tyrosine at EX/EM(intensity):224/355 nm (1413) and 278/352 nm (2942) Figure3B indicates the peaks of the spectrum of the mixture for 100 µg/L Tryptophan with 100 µg/L Tyrosine at EX/EM (intensity):226/355 nm (1421) and 278/352 nm (2860). Figure 3C indicates the peaks of the spectrum of the mixture for 100 µg/L Tryptophan with 150µg/L Tyrosine at EX/EM (intensity):224/349 nm (1501), 228/304 nm (1706), 276/310 nm (2000) and 276/351 nm (2942). Figure3D indicates the peaks of the spectrum of the mixture for 100 µg/L Tryptophan with 200 µg/L Tyrosine at EX/EM (intensity):224/349 nm (1504), 228/307 nm (2588), 276/307 nm (2721) and 278/349 nm (3054). Figure3E indicates the peaks of the spectrum of the mixture for 100 µg/L Tryptophan with 300µg/L Tyrosine at EX/EM (intensity): 226/340 nm (1831),228/307 nm (4230), 276/307 nm (3945) and 276/346 nm (3186). Figure3F indicates the peaks of the spectrum of the mixture for 100 µg/L Tryptophan with 400 µg/L Tyrosine at EX/EM (intensity): 226/307 nm (5313) and 276/307 nm (5292).
According to the peaks occurring at Ex/Em: 226/301 nm and 214/304 nm for the spectrum of tyrosine, we find that when the concentration ratio for tyrosine and tryptophan is below 150/100 nm, the target peaks of the spectrum of tyrosine disappear (Figure 3A, 3B) [17]. This can be attributed that tryptophan quenches the fluorescence of tyrosine. The target peaks occurring at Ex/Em: 222/349 nm and 278/352 nm for the spectrum of tryptophan are not affected by tyrosine in the spectrum of the mixture. The target peaks of tyrosine in the spectrum of the mixture appear more and more clearly by increasing the concentration ratio of tyrosine (Figure 3C, 3D, 3E and 3F).

Figure 4. indicates the simultaneous fluorescent spectra ($\Delta\lambda=70$ nm) of the mixing solutions for constant concentration 100 $\mu$g/L of tryptophan with various concentration 50, 100, 150, 200, 300, 400 $\mu$g/L of tyrosine. The intensity of the peak occurring at excited wavelength 220 nm increases by increasing the concentration of tyrosine. According to the data of Figure 4, we make two linear regression plots of the intensity of the peaks at excited wavelength 228 nm and 276-278 nm, with concentration of tyrosine (Figure 5).

![Figure 4](image.png)

**Figure 4. SFS diagram of constant tryptophan (100 $\mu$g/L) with varied tyrosine concentrations ($\Delta\lambda=70$ nm)**

Figure 5 indicates that the fluorescent intensity and concentration for tyrosine have a positive linear relationship at excited wavelength 228 nm and at excited wavelength 276-278 nm, the fluorescent intensity little decrease by enhancing concentration of tyrosine. This result can be explained that at excited wavelength 276-278 nm, the fluorescent intensity for tryptophan is much stronger than for tyrosine and the total fluorescence intensity of the mixing solution shows small changes by varying the concentration of tyrosine.
Figure 5. Linear relationship between specific fluorescent peak intensity and Tyr. concentrations at constant 100 µg/L of Trp. at both Ex=228 nm (R = 0.9889) and EX=276-278 nm (R = 0.9272)

4. Conclusions
1. The target peaks of the fluorescent spectrum of the single solution for tryptophan and tyrosine occur at Ex/Em: 222/349nm (1328), 278/352nm (2585) and Ex/Em: 226/301 nm (1270), 214/304 nm (1212), respectively.
2. The fluorescent intensity and wavelength of Tyrosine is higher than Phenylalanine due to the phenol group which has longer conjugated length than benzene ring.
3. when the concentration ratio for tyrosine and tryptophan is below 150/100, tryptophan quenches the fluorescence of tyrosine.
4. The fluorescent intensity and concentration for tyrosine have a positive linear relationship at excited wavelength 228 nm and at excited wavelength 276-278 nm, the fluorescent intensity little decrease by enhancing concentration of tyrosine.

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