Study on Ethanol-Water Cluster Types with Steady State and Time Resolved Fluorescence Spectrometer*

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Abstract: The steady state and time resolved fluorescence spectrum of ethanol-water solution has been studied. The three different emission bands located at 290 nm, 305 nm and 330 nm, respectively, and the three corresponding excitation lights with the absorption peaks of 265 nm, 280 nm and 236 nm have been measured respectively by the fluorescent spectra and the excitation spectra. The decay process of fluorescence intensity was detected and the curve was fitted by an exponential function. The lifetime parameters of different fluorescent photons were obtained by the numerical analysis procedure of deconvolutions. The characteristic of the steady state spectra and the three different lifetimes confirm the fact that there are three clusters in ethanol-water solution.

Key words: Fluorescent spectrum; Fluorescence time; Quantum yield; Ethanol-water cluster
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0 Introduction

The structural features of ethanol molecules in different phase states and the characteristics of the associated cluster molecule groups, which are generated by ethanol to water hydrogen bonding, have attracted great interest in the last decade. Nevertheless, there are still no reasonable accepted conclusions about the structure of ethanol molecules in liquid, solid, and especially in ethanol-water solutions[1-6]. In those studies, some methods have been used, such as X-ray absorption spectroscopy, X-ray emission spectroscopy and neutron diffraction analysis, but there is no reports about the fluorescence techniques. The fluorescence spectrum technology is a keen analysis method to study the molecular structure and light-material interaction[7-9]. Especially, the time resolved fluorescence spectrum is independent of the path of detecting rays, only relative to micro-environment, some information of molecular structure, steric and electronic environment and so on[10-12]. Comparatively, the technique is seldom used to investigate molecular clusters formed in ethanol-water solution.

In our previous study, ethanol-water solutions can emit fluorescence which is different from ethanol and water molecules respectively when irradiated by the UV-light[13-14]. One can conclude that hydrogen bonding of ethanol with water molecules, might form new cluster molecule groups with different structures by ethanol and water molecules. The combined numbers of the ethanol-water cluster molecule groups, which counted by the theory of analytical chemistry and organic chemistry, were investigated using light-induced steady state fluorescence spectroscopy. And the lifetime of the fluorescent photon has not been studied in detail. In this paper, the fluorescence lifetime of the emission photon has been detected and then analyzed by physics and mathematic theory in order to certify the molecular structural characteristic of the new ethanol- water clusters.

1 Experimental section

The Lifetime and Steady State Fluorimeter 900 (FLS900) combined with fluorescence lifetime & steady-state spectrometers made in Edinburgh Instruments Ltd. of UK were used in our experiments. The light source for steady-state excitation is a Xe 900, 450W Xenon arc lamp, with continuous spectral distribution from 190 nm to 2 600 nm. The wavelength of the excited-light is selected by the grating from 230 nm to 300 nm and the scan range of the emission spectrum is from

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300 nm to 500 nm. The fluorescence lifetime spectrometer, model nF900 (nanosecond flashlamp) manufactured by the same company is all-metal, triggered flashlamp, engineered with a fast switching thyratron, spark gap and charging resistor. A fluorescence lifetime spectrometer is based on the Time Correlated Single Photon Counting (TCSPC) technique. The excitation light is selected with the wavelength of 272 nm and the frequency of 40 kHz, counting 2 000 times at wavelength of fluorescent peak, and the gained kinetic curve of fluorescent decay will be deconvolved with exponential fitting.

The samples were tri-distilled water and HPLC ethanol with the concentration no less than 99.5%, which was produced by TEDIA Co. of USA. The aqueous solutions of ethanol-water mixture were prepared in the way of the total volume of ethanol and water solutions keeping fixedness for 3 ml, and the volume percent of ethanol solution changed such as: 100%, 90%… 10%.

2 Results and discussion

2.1 The steady state fluorescence spectrum

We have measured the steady state emission spectrum and the excitation spectrum of ethanol-water solution, which are shown in Fig. 1. There are three emission bands in the fluorescent spectrum of ethanol-water solution, which centres are located at 290 nm, 305 nm and 330 nm respectively. Because the 345 nm band is red-shifted along the excitation light increasing, it is not the fluorescence emission of the solution.

And the excitation spectrum of these fluroescent photons shown in Fig. 2 revealed that the solution emitting the fluorescence band of 290 nm is mainly due to absorbing the excitation light of 265 nm . When the solution absorbs the excitation light of 280 nm it can emit the fluorescence

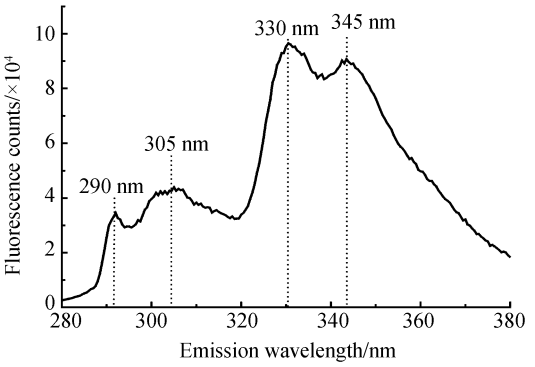
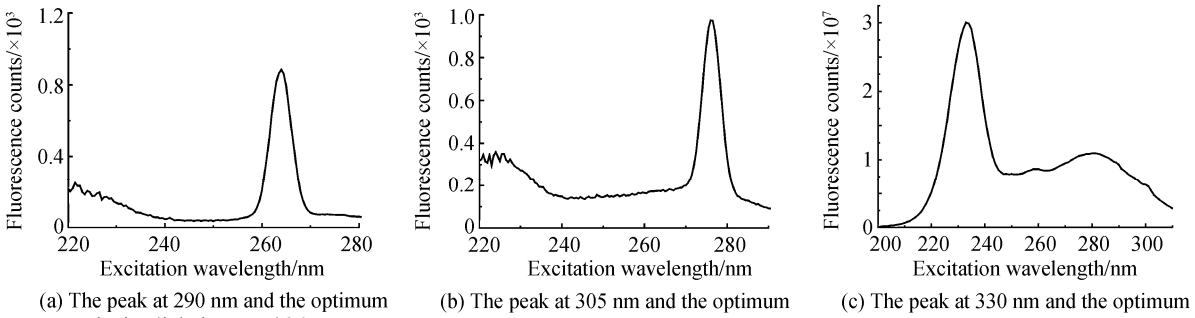


Fig. 1 The emission spectrum of ethanol-water mixture excited by the UV-light

photons at 305 nm. And the emitting of 330 nm is mainly due to absorbing the excitation light of 236 nm. The solution can absorb the excitation light from 265 nm to 280 nm and then emit these fluorescence bands. The fluorescence quantum yield of the excitation light of 236 nm is the highest among all excitation lights and about 30% quantum yield of the excitation lights from 265 nm to 280 nm . As the fluorescence spectrum and excitation spectrum reflect the local electronic structure of the various conformations, and can probe the unoccupied and occupied electronic states of the molecules, different absorption bands and emission bands are corresponding to different molecular structures. Absorption and emission spectra are sensitive to the energy shift by molecular clustering. That is to say, clustering of ethanol and water molecules creates new structure and energy level in this spectral range. We find that the ethanol-water mixture has three different absorption bands and three different emission bands, which is different from in the pure ethanol and water liquid. It can be concluded that ethanol and water molecules get together to form molecular clusters and emit different fluorescence photons .



(a) The peak at 290 nm and the optimum excitation light is around 265 nm

(b) The peak at 305 nm and the optimum excitation light is 280 nm

(c) The peak at 330 nm and the optimum excitation light is 236 nm

Fig. 2 The excitation spectrum (ES) of the three fluorescence emission peaks

The three types clusters homologize the three different emission bands.

2.2 The intensity decay process of the emission band

The decay curve of the fluorescence intensity at 50% and the computer fitting data are shown in Fig3.

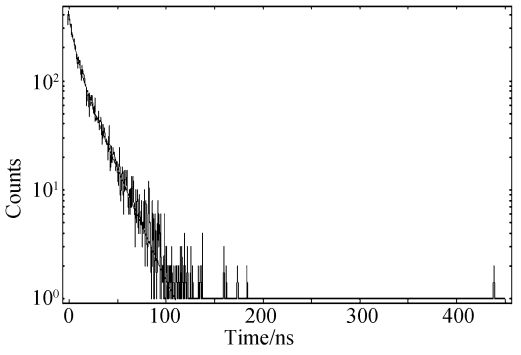


Fig. 3 Exponential fit curves of the decay Process, the excitation light is 236 nm and the emission peak is 330 nm

If there is only one component emitting fluorescence photons, the exponential curve of the decay process will be linear. The negative reciprocal value of this linear slope coefficient is the lifetime of the fluorescence photon. When the illuminant material includes more than two components, the exponential curve will no longer be linear. As different lifetimes correspond to different slopes and the logarithm decay curve bends accordingly. The curve may divide into several parts and each part is fitted to a linear respectively. If the linear comprise several slope rates, there are many kinds of materials emit fluorescence. From Fig. 3, we can see that the logarithmic decay curve is not linear any longer. It is proposed that there is more than one kind of substance emitting fluorescent photons with the wavelength 330 nm in the mixed solution. According to the three lifetime values, it can conclude that there are three components absorb the excitation light of 236 nm and emit fluorescence photons respectively. When the volume percent is 50%, the decay curve is composed of three parts with three different lifetimes, which means there are three different fluorescent molecules. This result is consistent with the analyses from steady state fluorescence spectrum. Namely, from the steady state spectrum we can find all the three fluorescent substances can absorb the excitation light at 236 nm, and emit photons at 330 nm, of different lifetime about 20.67 ns , 6.83 ns , and 2.72 ns, respectively.

If there are more than one kind of fluorescence substances and they are mixed together, each kind of component has the possibility to absorb the excitation light and emit fluorescence photons, using single photon count technique and combining with data analytical soft, the proximate and content analysis can easily be done about the mixture. The relative concentration of different componentc_1, c_2, c_3, the relative fluorescence intensity of different component as a percentage ϕ_1, ϕ_2 , ϕ_3 and the average lifetime of the entire decay process $\langle \tau \rangle$ is calculated respectively as in Ref [15].

By the formula the relative content percents of the three fluorescent substances at 50% ethanol mixture are

$c_1 = 38\%$, $c_2 = 27.8\%$, $c_3 = 34.2\%$

where here c_1, c_2, c_3 indicate the component with the longest, longer and the shortest lifetime, respectively.

The percent of three substances′ fluorescence intensity occupying in the total are

$\phi_1 = 75.5\%$, $\phi_2 = 20.0\%$, $\phi_3 = 4.6\%$

The total fluorescent lifetime is $\langle \tau \rangle = 22.4$ ns. From the experimental data, we can see that the content of fluorescent the substance 1 is almost equal to the substance 2, but the fluorescence intensity is more three times than the latter, and that can explain why the quantum production rate of fluorescent the substance 1 is higher than the substance 2. The substance 3 is the lowest relatively. According to the steady state fluorescence spectra, the substance absorbing the light at 236 nm and emitting fluorescence photons at 330 nm may be the substance 1.

In this experiment, we have carried on the test separately to all solutions with different ethanol volume percent, just the same as 50% solutions, each of them can obtained the content of three types of substances, the fluorescence intensity percent and the changing situation of total fluorescent lifetime along with the ethanol volume ratio. The results are shown in the following table 1 and in Fig. 4.

Table 1 Calculate result of different volume percents ethanol-water solution

Volume percent of ethanol	$\langle\tau\rangle$ns	c_1%	c_2%	c_3%	ϕ_1%	ϕ_2%	ϕ_3%	Y_{F1}/Y_{F2}	Y_{F1}/Y_{F3}
10%	16.8	23.8	76.2		42.2	57.8		3.0	
20%	17.6	20.0	49.0	31.0	43.5	51.5	5	2.0	13
30%	18.5	19.9	42.0	37.9	43.8	47.3	8.9	1.8	9.3
40%	22.1	35.2	29.1	35.8	69	24.7	6.3	2.3	11
50%	22.4	38.0	27.8	34.2	75.5	20.0	4.6	2.1	10.8
60%	22.5	43.6	27.7	28.7	69.8	24.3	5.9	2.4	10.6
70%	20.5	41.4	19.5	39.1	68.8	24.5	6.6	2.1	10.3
80%	18.6	36.6	27.1	36.3	72.9	17.8	9.3	3.0	7.8
90%	15.1	3.3	53.4	43.3	7.7	79.6	12.8	1.6	7.9
99.7%	11.9		52.8	47.2		80.9	19.1		

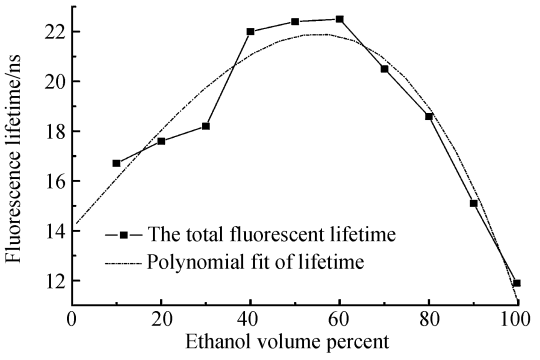


Fig. 4 The relationship between the total fluorescent lifetime and the ethanol volume ratio

The fluorescence quantum productive rate is defined as the ratio of the emitting to the absorbing quanta which is called the fluorescence efficiency. The fluorescence quantum production rate is an important illumination parameter of fluorescent substance, which has the vital significance in the spectral analysis. Liquid illuminant's fluorescence intensity F is not only relative to the material fluorescence quantum production rate Y_F, but also to the solution concentration c and molar absorption coefficient ε, namely

$$F=2.3Y_F I_0 \varepsilon bc \tag{1}$$

In order to compare the fluorescence quantum yields of the three components, the quantum yield ratios of three substances are calculated by Eq. (1). Y_{F1}/Y_{F2} (the ratio of the substance 1 to the substance 2) and Y_{F1}/Y_{F3} (the ratio of the substance 1 to the substance 3) are shown in the table. From the table we can see, in the higher and lower ethanol volume percent solutions, the ratios are very variable, only in the solutions of 50% to 70%, Y_{F1}/Y_{F2} is changeless near to 2 and Y_{F1}/Y_{F3} is about 10. According to the Ref[14], 60% solution is easy to form the substance 1, which emission peak is 330 nm and the excitation light is 236 nm, the experiment data is the nearly same as the intrinsic lifetime. In the solutions of 30%, 80%, it is easy to form the other two substances, and the detected centre is far away from the emission peaks and the excitation light of the substances 2 or 3, so the measurement data deviates accordingly.

The relationship between the fluorescence quantum production rate and the lifetime is

$$Y_F=\tau/\tau_F \tag{2}$$

The sign τ indicates the fluorescent lifetime, and τ_F indicates the molecular inherent lifetime.

By formula (1) and (2), we obtained that

$$F=2.3\varepsilon bcI_0\tau/\tau_F \tag{3}$$

According to formula (3), when the inherent lifetime is definitive, the fluorescent lifetime is directly proportion to the fluorescence intensity, which means their change rule is similar. In the research of steady state fluorescence spectrum which has been detailed studied in the Ref[14], the fluorescence intensity of the emission band centered at 330 nm increases as the solution concentration increasing at first, when it comes to the maximum at 60% (the ethanol volume percent), it reduces gradually as the volume percent increasing sequentially. The reason is that the concentration of the substance 1 comes to the maximum in 60% ethanol-water solution. And the excitation wavelength is 236 nm, which is the optimal excitation of fluorescence photon with the wavelength of 330 nm, so the fluorescence quantum yield is the biggest. Hence, the fluorescence intensity comes to the peak. About the fluorescent lifetime the similar rule presents too, the fluorescent lifetime increases firstly and then decreases as the solution density changing, and the longest lifetime appears at 60%. From Eq. 2, fluorescence quantum production rate is linear to

the fluorescence lifetime, so when the volume percent of ethanol-water is 6:4, in which the fluorescence intensity comes to the peak, the lifetime comes to the peak too. The two experiments' result of the steady state and time resolved inosculates nicely. In 60% ethanol-water solution, the ratio of the molecular number of ethanol and water is about 1:2, that is to say one ethanol and two water molecules cluster together to form a new structure, which is corresponding to the substance 1 with the emission peak of 330 nm and the longest lifetime of 20 ns.

3 Conclusion

According to the steady state and time resolved fluorescence spectrometer, it can be concluded that when ethanol solutions mixed with water solutions, the molecules get together to form some new molecular clusters that could emit different fluorescence photons in subsequent experiment. Different absorption bands and emission bands are corresponding to different molecular structures. There are three components in ethanol-water mixed solutions. The fluoerscence intensity decay process indicates that the three components have different lifetimes. The longest lifetime is corresponding to the emisison of 330 nm, which has the largest fluorescence intensity when irradiated by 236 nm excitation light. In the 60% solution, the possible structure is one ethanol molecule and two water molecules cluster. Our results show that most water and ethanol molecules in the solution persist in structures similar to the ones found in the pure liquids. The clusters molecular structure is on the way of research continually.

References

[1] TALON C, RAMOS M A, VIEIRA S. Low-temperature specific heat of amorphous, orientational glass, and crystal phases of ethanol[J]. *Physical Review B*, 2002, **66**: 012201-1-012201-1-012201-4.

[2] MATIC A, MASCIOVECCHIO C, ENGBERG D, *et al*. Crystal-like nature of acoustic excitations in glassy ethanol[J]. *Physical Review Letters*, 2004, **93** (14): 145502-1-145502-4.

[3] SUROVTSEV N V, ADICHTCHEV S V, WIEDERSICH J, *et al*. Fast relation in the structural glass and glassy crystal of ethanol and cyano cyclohexane: a quasielastic light scattering study[J]. *J Chem Phys*, 2003, **119**(23): 12399-124084.

[4] GUO J H, LUO Y, AUGUSTSSON A, *et al*. Molecular structure of alcohol-water mixtures [J]. *Physical Review Letters*, 2003, **91**(15): 157401-1-157401-4.

[5] TAYLOR R S, SHIELDS R L. Molecular-dynamics simulations of the ethanol liquid-vapor interface[J]. *J Chem Phys*, 2003, **119**(23): 12569-12576.

[6] CRIADO A, JIMENEZ R M, CABRILLO C, *et al*. Rotational dynamics in the plastic-crystal phase of ethanol: Relevance for understanding the dynamics during the structural glass transition[J]. *Physical Review B*, 2000, **61**: 12082-1-12082-12.

[7] XU X D, ZHAO Z W, SONG P X, *et al*. A study on fluorescence characteristics of Yb : YAG crystals [J]. *Acta Photonica Sinica*, 2004, **33**(6): 697-699.

[8] WANG C L, QIAN L, FAN D W, *et al*. Spectral research on delayed fluorescence of chloroplasts at different concentration [J]. *Acta Photonica Sinica*, 2005, **34**(7): 1028-1031.

[9] LI R Q, LIU Y, GE L X, *et al*. Study on blood cells disintegration by laser included fluorescence spectrometry[J]. *Acta Photonica Sinica*, 2006, **35**(3): 398-401.

[10] BELFIELD K D, BONDAR M V, PRZHONSKA O V, *et al*. Steady-state spectroscopic and fluorescence lifetime measurements of new two-photon absorbing fluorene derivatives[J]. *Journal of Fluorescence*, 2002, **12**(3): 449-454.

[11] CHEN G Z, HUANG X Z. Fluorescence analysis. Beijing: Science Press, 1990: 121-129.

[12] MAO Y L, DING F, GU Y. The influence of concentration of Yb～(3+) ions on luminescence and fluorescence lifetime in Yb : YAG crystals[J]. *Acta Photonica Sinica*, 2006, **35**(3): 365-368.

[13] LAN X F, LIU Y, SHEN Z H, *et al*. Fluorometic determination of ethanol solution[J] *Acta Photonica Sinica*, 2003, **32**(11): 1371-1374.

[14] LIU Y, PENG C D, LAN X F, *et al*. Fluorescence spectrum characteristics of ethanol-water clusters [J]. *Acta Physica Sinica*, 2005, **54**(11): 5455-5461.

[15] LIU Y, LI R Q, SHEN Z H, *et al*. The experimental study on the fluorescence lifetime of ethanol-water mixture [J]. *Chieses Optics Letters*, 2005, **3**: S88.

用稳态和时间分辨荧光光谱研究乙醇－水分子团簇的可能类型

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摘 要：研究了紫外光照射乙醇－水混合溶液的稳态和时间分辨荧光光谱. 通过检测其荧光光谱和激发光谱，得到了稳态发射光谱的三个荧光峰，峰值分别位于 290 nm、305 nm、330 nm，相应的最佳激励光分别为 265 nm，280 nm 和 236 nm. 在荧光光谱峰值波长处分别监测其荧光强度随时间的衰变过程，将获得的荧光衰减动力学曲线采用指数拟合并进行解卷积处理获得不同荧光光子的寿命值. 乙醇－水溶液稳态光谱的特点和三个不同的荧光寿命都表明了溶液中含有三个不同的生色团，分析认为乙醇和水分子间通过氢键作用形成了不同结构的团簇分子.

关键词：荧光光谱；荧光寿命；荧光量子产率；乙醇－水团簇分子

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