Derivative fluorimetry analysis of new cluster structures formed by ethanol and water molecules

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Received September 12, 2008

The ultraviolet (UV) light excited fluorescence spectra of ethanol-water mixture with different concentrations are investigated by derivative fluorimetry. It is found that there are 8 types of luminescent cluster molecules, formed by ethanol and water molecules in different ways, existing in the solution. The peak wavelengths of all these clusters' fluorescence spectra are measured and their contents are obtained by measuring the peak values in the second derivative fluorescence spectra. The spectra corresponding to the 8 types of clusters are obtained by Gaussian decomposition. It is found that two kinds of cluster molecules whose peak wavelengths are 330 and 345 nm have an optimal excitation wavelength located at (236 ± 3) nm. This research contributes to the study of ethanol-water cluster structures and their physical and chemical characteristics.

OCIS codes: 300.6390, 300.2140, 300.2530.

doi: 10.3788/COL20090702.0159.

The interaction between ethanol molecules and biological tissues has been subjected to numerous investigations. Upadhya showed that ethanol molecules had a notable impact on nerve cells through changing their main ingredients[1]. Some researches indicated that ethanol molecules could inhibit cell proliferation and increase apoptosis of cancer cells[2]. Later, de la Monte *et al.* studied the dog embryos exposed in the ethanol environment and found that puppies' brain growth was incomplete[3]. However, the microcosmic mechanism of the interaction between ethanol molecules and biological tissues is not so clear. To study the interaction between the ethanol molecules and the relatively simpler water molecules can offer help to this issue. Some investigators studied pure ethanol under room temperature and measured 10 of 21 partial structures liable to be affected. The results indicated that the molecules formed zigzag chain structures linked by hydrogen bonds in pure ethanol liquids[4−7]. Matic *et al.* analyzed the data of X-ray diffraction (XRD) experiment on liquid ethanol molecules and proposed there were monomers, tetramers, pentamers, and hexamers, linked by hydrogen bonding, existing in the liquid ethanol[8−10]. However, a convincing description of the details of the association is lacking. When mixed with water, ethanol will dissolve in a very complicated way. It is even harder to detect the structure of the molecules. Liu *et al.* studied the solution with fluorescence spectrometry and obtained some new information, but the definite result has not been clear yet.

Through analyzing the fluorescence spectra, Liu *et al.* proposed that 3 kinds of new cluster molecules were formed by association reactions of water molecules and ethanol molecules in different ways[11−13]. But the band overlapping which may bring disturbance to the analysis always exists in the wide band structure's spectral line, so the information may be lost if we analyze the original spectrum directly. The derivative fluorimetry can resolve the characteristic spectrum effectively and discriminate the slight change of the spectrum. It is very useful to distinguish the band overlapping of the mixture spectrum, increase the definition of secondary spectra, and measure the weak preshoulders[14]. This method is used to analyze the spectra of ethanol-water solution in this letter, and the information of luminescent clusters could be obtained. And then Gaussian decomposition method was used to obtain the emission spectrum of each cluster. The results could provide experimental and theoretical basis for further study.

The Lifetime and Steady State Fluorimeter 900 (FLS900) combined with fluorescence lifetime and steady-state spectrometers (Edinburgh Instruments Ltd., UK) was used in our experiments. The light source was the Xe-900 lamp with tunable luminescence wavelength from 190 to 2600 nm and power of 450 W. The detector scanning range was from 200 to 900 nm.

The materials used in the experiment were tri-distilled water and high purity ($\geq 99.5\%$) ethanol (TEDIA Ltd., USA). The two materials were mixed in different proportions to form ten solutions of different concentrations, the volume ratios of ethanol to water in the solutions were 9.5:0.5, 9:1, 8:2, 7:3, $\cdots$, 2:8, 1:9, respectively.

The ultraviolet (UV) light excited fluorescence spectra of the ten solutions were measured with fluorescence spectrometer. The excitation wavelength was 236 nm and the emission scanning range was $280 - 400$ nm.

The fluorescence spectra of different concentration mixtures excited by UV light are shown in Fig. 1. All the spectra show that there are 4 emission peaks located at 292, 304, 330, and 345 nm, respectively. But it is hard to obtain more detailed information because of the overlapping of the spectra.

The spectra shown in Fig. 1 were filtered with the fast Fourier transform (FFT) low pass filter to remove the

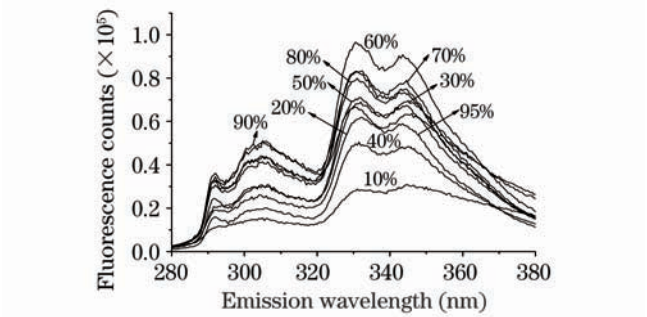

Fig. 1. Fluorescence spectra of the solutions excited by 236-nm UV light. The volume percentages are given for ethanol in the solutions.

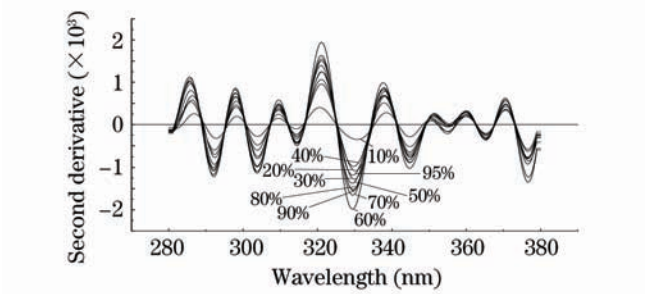

Fig. 2. Second derivative curves of the original spectra.

impacts of the noise signals, and the Savitzky-Golay method was used to smooth the filtered spectra. Then the derivative spectra were obtained through the second derivate calculation to the smoothed data (see Fig. 2). The different curves represent the second derivative spectral lines of different concentration solutions. Each derivative spectral line has 8 minima which are located at around 292, 304, 314, 330, 345, 355, 365, and 377 nm, respectively. The 8 positions are approximately identical in the ten spectral lines with the maximum variance of 1 nm. The minimum of the second derivative spectrum corresponds to the maximum of fluorescence emission, so each original spectrum has 8 fluorescence peaks, and one can conclude that there are 8 kinds of luminescence structures existing in each solution. The fluorescence peaks of different kinds of structures are the 8 minima given above. In addition, the second derivative spectrum intensities of solutions with different concentrations are distinct at each minimum. Because the derivative value is proportional to the content of the corresponding luminescence at a certain wavelength position[14], it is concluded that the concentration of the solution affects the quantities of the cluster molecules.

The relation between the emission intensity at specific wavelengths and the solution concentration is shown in Fig. 3, where the intensity is represented by the negative second derivative values of the original spectra. The 8 different curves represent the 8 peak positions given above.

It can be seen from the figure that the derivative values of the five spectral peaks located at 292, 314, 330, 345, and 365 nm reach maximum when the solution concentration is 60%, and these values will decrease when the concentration deviates from this value, the more deviation, the less the intensity values will be. But interesting things occur when the concentrations are 30% and 70% − 80%, where the derivative values have a sharp

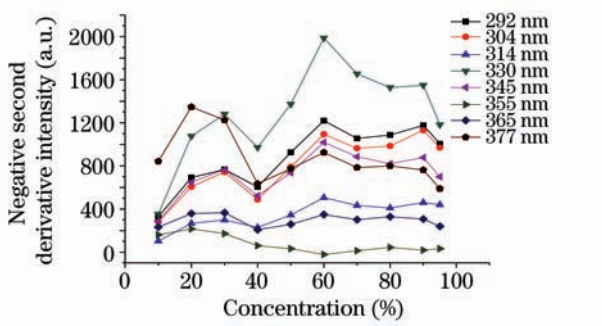

Fig. 3. Negative second derivative values of the fluorescence spectra.

reduction and two valleys are formed in the two places, and the 304-nm curve has the similar behavior. The 355-nm curve reaches maximum when the concentration is 20% and has a valley at 60% concentration. The 377-nm curve also has a maximum at 20% concentration, but has two valleys when the concentrations are 40% and 70%.

It is demonstrated that the contents of the 6 kinds of cluster molecules whose fluorescence peak wavelengths are 292, 304, 314, 330, 345, and 365 nm reach maximum when the solution concentration is around 60%. And the other 2 kinds of cluster molecular contents reach maximum around the concentration of 20%. The fluorescence emission quantum yield will decrease when the solution concentration deviate the certain value. This is because the low concentration induces the low contents of luminescence clusters, and too high concentrations also can induce the decrease of the fluorescence emission quantum yield, which should be attributed to the concentration effect including inner filter effect and the formation of excimer[15].

The 314-, 355-, and 365-nm curves are more planar compared with the other 5 curves, which states that the contents of the corresponding 3 kinds of clusters are less sensitive to the solution concentration change compared with the others.

Gaussian decomposition was applied to the fluorescence spectra based on the 8 peak wavelengths obtained by the second derivative fluorimetry method, and the results are shown in Fig. 4. The solid line in the figure represents low-pass spectra, and the dashed line shows the multi-peak fitted spectra, and these two spectral lines nearly superpose with each other. The dotted lines are fluorescence spectra of the 8 kinds of cluster molecules, defined as No. $1-8$ element spectra in turn from blue to red range, thus the components of the original spectrum can be seen clearly. This can give detailed information of the 8 kinds of cluster molecules and also provide

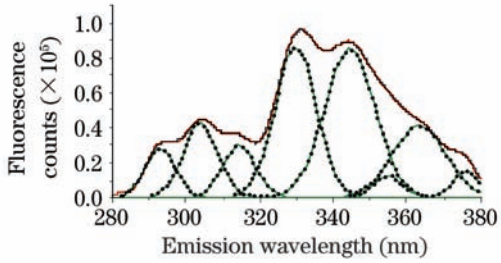

Fig. 4. Spectra after Gaussian decomposition for the solution with 60% concentration.

evidences to further investigation.

There are differences between the fluorescence peaks of the elements and the fitted line. It is caused by the overlapping of the spectral lines. Taking the No. 4 element for example, its peak is red-shifted and the peak value is also increased because of the effect of the No. 5 element, and this may carry errors to spectral analysis. Utilizing Gaussian decomposition method can resolve this problem.

It can be seen from Fig. 4 that the fluorescence spectrum has stronger intensities in the No. 4 and No. 5 peaks, corresponding to the elements with 330- and 345-nm peak wavelengths, when excited by the 236-nm UV light (we here show the figure of 60% concentration solution only and the others have the same situation). We can conclude that the corresponding two kinds of cluster molecules have stronger absorption to the excited light than the others. The peak with maximum intensity will change as the excitation wavelength alters[11]. The other 6 kinds of cluster molecules can also absorb the 236-nm light and emit photons, but the absorption is slight. Utilizing this characteristic, we can obtain the respective main absorption wavelength from further experiment.

In conclusion, the fluorescence spectra of ethanol-water mixtures were analyzed using the second derivative fluorimetry method and it was found that there were 8 kinds of luminescence structures existing in the solutions when excited by the incident light with the wavelength of 236 nm. And the fluorescence spectral peaks were located at 292, 304, 314, 330, 345, 355, 365, and 377 nm, respectively. The two kinds of clusters whose fluorescence peaks are 355 and 377 nm have maximum contents when the solution concentration is 20%, while the other 6 kinds have maximum contents when the concentration is 60%. And the clusters whose fluorescence peaks are 292, 304, 330, 345, and 377 nm are more sensitive to the solution concentration compared with the other 3 ones. The clusters whose center emission wavelengths are 330 and 345 nm have a main absorption wavelength at (236 ± 3) nm, and this wavelength for the other clusters needs further investigations. Using Gaussian decomposition method we can educe that the elements of original spectra and more detailed information of the clusters, such as spectral intensity, half width, and accurate peak wavelength, can be obtained. This method can be helpful to the further research.

This work was supported by the Natural Science Foundation of Jiangsu Province under Grant No. BK2007204.

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