Research of the mechanism of acetic acid fluorescence quenching *

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Abstract : Analyses of the mechanisms of acetic acid emitting fluorescence and fluorescence quenching have been made in theory. The results indicate that acetic acid solutions can emit visible fluorescence when induced by 253. 7nm UV light , and fluorescence quenching occurs along with the alteration of solution concentration. It is thought that fluorescence dynamic quenching process could take into account the intersystem transition of (n , *) to (, *) , and the quenching constant is calculated in this paper. Investigation on the native fluorescence spectrum and its characteristics will contribute to the study of other molecules fluorescence spectra when acetic acid serves as solute , hydrolysis catalyst and food additive.

Key words : acetic acid ;fluorescence spectrum ;fluorescent quenching ;ultraviolet light-induced

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253. 7nm   ,   ,

——   (n , *)   (, *)   ,

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Instruction

Acetic acid has been extensively studied as an organic solvent , where it is mostly used as a hydrolysis catalyst , fluorescence quenching , and food additive , and the characters of other molecules fluorescence have been well studied when acetic acid serves as an additive. It is well known that acetic acid plays an important role in colposcopic diagnostics in order to detect some cervical abnormalities , making them white and more noticeable by the human eye. Zelenchuk studied about fluorescence spectra of freshly excised cervical tissue and the data in this study from non-neoplastic cervical tissue reveal changes after the application of acetic acid[1]. Harris research demonstrated that in the case of acetic acid fixation sperm visualization became easier and could make the difference in fluorescence coloration between sperm and cell cytoplasm[2]. Acetic acid also has been well studied when it serving as food additive. Acetic acid is a potential inhibitor of yeast growth and survival[3]. Its inhibition mechanism was generally assumed to involve the passive diffusion of acetic acid in its undissociated form across the plasma membrane of the cell. And the reason of causing intracellular acidification was that the undissociated acetic acid dissociates because of the higher intracellular pH[4,5]. When serving as fluorescence quenching , acetic acid quenching mechanism has been studied. Girousi's found that formaldehyde

could emit a strongly fluo-rescence in investigating fluoro-metric determination of formaldehyde and fluorescence was quenched by acetic acid. Quintero *et al.* pointed out that fluorescence dynamic quenching process could represent a serious inconvenience in analytical fluorimetric techniques taking into account acetic acid being commonly used as a buffer and additional fluorescence quenching by H$^+$ ions could be involved in acid aqueous mediums[6]. But in all of these studies, acetic acid was considered as a non-fluorescence emission matter.

Despite an extensive research on acetic acid when serving as solvent, we have never found any publication describing acetic acid native fluorescence emission. The main reason is that acetic acid doesn't absorb visible light, that is to say, it is optic transparent within this wavelength, so it has no effect on other mo-lecules emitting fluorescence. However, acetic acid can absorb ultra-violet light; few researchers study whether acetic acid can emit fluorescence after absor-bing these UV lights. Almost in all researches when acetic acid severed as solvent, it is widely noted that fluorescence quenching is caused by acetic acid with dynamic and static processes. Therefore, it is very urgent to clarify the fluorescent character and the mechanisms of acetic acid fluorescence quenching. In this paper, fluorescence spectra of acetic acid are investigated and the dependence of fluorescence intensity and quench-ing phenomenon are presented.

1   Materials and experimental results

The experimental apparatus used to obtain the fluorescence and absorption spectra from different acetic acid aqueous solutions have been detailed in our earlier paper[7]. All the reagents are with high purity. In the spectrofluorimetric measurements triply distilled water is used to dilute the sample to form different concentration solutions. In order to test fluorescence spectra character of acetic acid solutions with different concentrations, the sample is diluted to form from several thousandths till to several millionths. Then test the fluorescence intensity al-teration and the degree of fluorescence quenching effect respectively.

According to our early research, the fluorescence in-tensity changes as the sample concentrations alter, fluo-rescence quenching occurs in some solutions and the fluo-rescence intensity is so sensitive to the acetic acid concen-tration that fluorescent quenching occurs now and then.

2   Discussions

According to the experimental results given above we can conclude that acetic acid is able to radiate fluores-cence when induced by 253.7nm ultraviolet light. When the exciting light transmits through the acetic acid solu-tion, the energy of exciting light mostly converts fluores-cence photon energy. Acetic acid molecule enables its primary fluorophore to absorb the energy of exciting light and then transfer it into fluorescence photon energy. Based on the principle of fluorescence emission, acetic acid molecule has fluorophore. In analytical chemistry, chromophore group is the undersa-turated part of a molecule, which can absorb UV-VIS light and bring elec-tron transitions. The chromophore in acetic acid molecule is C = O[8] which has several types of electron transition such as *, *, n * and n * (See Fig. 1).

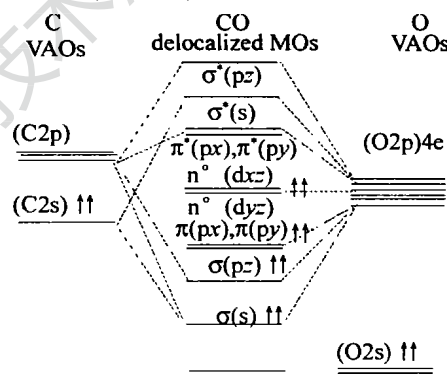

Fig. 1   Delocalized MO energy level diagram for C = 0

Among these energy level transitions, the n *, which is from nonbon-ding electron namely n electron to anti-bonding orbital *, must absorb the lowest photon ener-gy. Because electron transitions of *, n * and * must absorb exciting light with wavelength less than 200nm, we can conclude that the sample excited by 253. 7nm UV light results in n * electronic transition in chromophore C = O, that is to say, the n electrons in ground state absorb the photons energy and jump to excit-ed state of * orbit. In order to return to the ground state, the unstable electrons in excited state, losing its excess energy usually in the form of heat, transit to lower rotational and vibrational levels of excited state, and then return to the ground state and radiate fluorescence. Therefore the wavelength of the emitted fluorescence is

longer than the exciting light. From the diffe-rent lower levels of the excited state, the excited electrons return to the ground state and emit fluorescence photon with differ-ent energy, thus the broadening fluorescence spectrum peak around 395nm.

To understand the effect of the quenchers on the flu-orescence of the acetic acid, the data are analyzed con-ventionally by the Stern-Volmer equation and dynamic quenching process[9]:

$$I_0/ I_q = 1 + k_{SV}[Q] \qquad (1)$$

Where I_0 and I_q are the unquenched and quenched fluo-rescence emission intensities respectively; k_{SV} is the Stern-Volmer constants.

The first excited singlet state of carbonyl compound is(n, *) and in this case the quantum yield of S_1 T_1 intersystem transition can be increased. Because n * transition is partially forbidden, the antitransition process of * n is also partially forbidden. As the result, the life in the first excited singlet state(n, *) is longer than that in triplet state($ $, *) and the probability of intersys-tem transition of singlet-triplet is increased. Therefore, the first excited singlet states of acetic acid are very easy to transform to triplet and thus fluorescent quenching is easy to occur. According to the hypothesis of constant states, the concentration of excited-state molecule$^1M^*$ keep unvaried after being illuminated by excited light, and fluorescent quenching process caused by singlet-triplet transition are presented as follows:

$$M + h \quad ^1M^* \quad \text{(absorption process)} \qquad (2)$$
$$^1M^* \quad M + h \quad \text{(fluorescence process)} \qquad (3)$$
$$^1M^* \quad ^3M^* \quad \text{(intersystem transition)} \qquad (4)$$

The concentration$^1M^*$ with no fluorescent quenching is denoted as$[^1M^*]^0$ when singlet-triplet transition doesn't occur:

$$[^1M^*]^0 = \frac{I_a}{k_f + k_{ic} + k_{isc}} = \frac{I_a}{k_f + \quad k_i} = \frac{I_a}{k_f + k_{ic}} \qquad (5)$$

Where I_a is absorption speed or molecule activation creat-ed-speed, k_f is fluorescent speed constant, k_{ic} and k_{isc} are the constant of inner transform and intersystem transition speed. In the case of fluorescent quenching the singlet-

triplet transition k_{isc} is maximal and $[^1M^*]$ is:

$$[^1M^*] = \frac{I_a}{k_f + k_{ic} + k_{isc}} \qquad (6)$$

$\phi_f^0 = \frac{k_f[^1M^*]^0}{I_a}$ and $\phi_f = \frac{k_f[^1M^*]}{I_a}$ represent the fluores-cent quantum yield with and without existence of fluores-cent quenching respectively. Based on the above rela-tions, they are described as the following equation:

$$\phi_f^0 = \frac{k_f}{k_f + k_{ic}} \qquad (7)$$

$$\phi_f = \frac{k_f}{k_f + k_{ic} + k_{isc}} \qquad (8)$$

The ratio of ϕ_f^0/ ϕ_f is described as:

$$\frac{F_0}{F} = \frac{\phi_f^0}{\phi_f} = \frac{k + k_{ic} + k_{isc}}{k + k_{ic}} = 1 + \frac{k_{isc}}{k + k_{ic}} \qquad (9)$$

It can be obtained from Eq. (6) that the quenching con-stant k_{SV} is:

$$k_{SV} = \frac{k_{isc}}{k + k_{ic}} \qquad (10)$$

Based on relation (9), we can calculate the quenching constants in different concentration series solutions, which are and listed in table 1, and it is found that k_{SV} decreas-es with the decrease of the acetic acid solution concentra-tion.

Table1 k_{SV} of different concentration series

different concentration series	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
k_{SV}	1.83	1.46	1.08	1.04	0.76

Based on the results shown in the reference [7], in some acetic acid solutions when fluorescence quenching occurs the ratio of $F_0/ F = 2.81$, ($F_0/ F - 1$) is equiva-lent to the quenching constants of k_{SV} the concentration series of 10^{-2}. As to the other concentration series the ratio of ($F_0/ F - 1$) is also similar to the quenching con-stants k_{SV}. So we can conclude that the theoretical arith-metic of k_{SV} coincides with the experimental results.

3   Conclusions

From experiment results and above discussion, we can draw the conclusions:

Acetic acid solutions can absorb 253.7nm UV light and emit fluorescence with the peak wavelength at 395nm. Fluorescence intensities change with the solution concentrations, and fluorescent quenching occurs

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3.2

OE   3
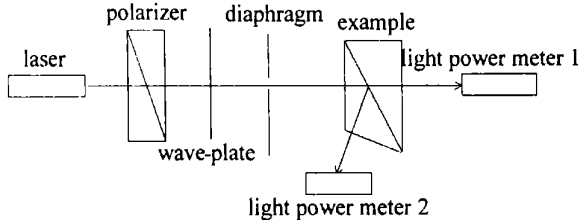
Fig.3   Measurement setup of intensity splitting ratio

15 ;

633nm   /4

(He-Ne

) ,   ,

1   2   o ,e

I_o I_e ,   (10)

4

4   C   ,   D

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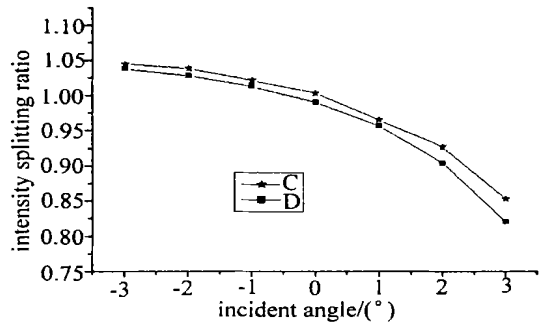;   OE   ,

Fig.4   The changeable relation of intensity splitting ratio with the incident angle

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0) ,e ,o   (I_e/I_o)   1 ;

, $I_e/I_o > 1$;   , $I_e/I_o < 1$

[1]   ,1984 ,10(4) :49 51.
[2]   ,   , *et al*.   ,1994 ,20(2) :
        50 53.
[3]   ,   , *et al*.   ,1994 ,18(5) :310 312.
[4]   ,   , .   ,1993(2) :46 47.
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in some acetic acid solutions. The quenching mechanism is owing to singlet-triplet transition and k_{SV} of different concentration series is calculated respectively. Even in very diluent solutions , acetic acid can emit strong fluorescence. The reason is that acetic acid mole- cules form a kind of planar and rigid structure joined by hydrogen bond and this structure enhances fluorescent emission.

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