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ORIGINAL ARTICLE

Spectroscopic Study of Aggregation of Carbazole Units

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Abstract

Several fluorescence spectral features in aggregation of carbazole unites have been reported in previous study. Unfortunately, the influence of inner filter effect has not been paid enough attention. In this work, aggregation of carbazole unites resulting from increasing concentration or decreasing solvent affinity, was investigated by fluorescence spectroscopy. Through comparison between the spectra of two aggregation processes, it is found that only intensity ratio of 0–0 transition to 0–1 transition in excitation/emission spectra is sensitive to aggregation states. There is an obvious decrease in that intensity ratio upon aggregation. On the other hand, other spectral features observed in previous research such as intensity decrease in $S_0 \rightarrow S_2$ transition and emission quenching in concentration induced aggregation process, should be induced by inner filter effect.

Keywords Fluorescence · Spectral feature · Aggregation · Carbazole

Introduction

Fluorescence of organic compounds is of great interest due to its wide application in various fields such as probes, sensors and bio-imaging. [1–4] However these applications are strongly dependent on close connections between emission and the microenvironment of organic compounds such as polarity or aggregation states. Either emission spectra or total intensity might be a sensitive indicator. For instance, pyrene is widely used as probes for micelle formation because ratio I_1/I_3 (the intensities of the first and third vibronic peaks in emission spectra) is sensitive to polarity of microenvironment. [5, 6] AIE compounds have also been widely used in bioimaging and detection of biomolecules [7, 8] relying on an intense increase in emission intensity upon aggregation [9, 10]. Therefore, establishment of the relationship between fluorescence spectra and aggregation states is important for further applications.

Carbazole-based compounds are of scientific and industrial interest [11–14] and their fluorescence has attracted considerable attention for a long time. [15–21] Most researches focused on fluorescence of molecules and seldom involved the fluorescence of aggregates and aggregation process. A concentration induced aggregation of carbazole unites has been investigated in a carbazole labeled polymer system. [22] In previous work three spectral features were summarized based on the concentration induced aggregation process. The first is a decrease in proportion of $S_0 \rightarrow S_2$ transition according to a red shift in excitation spectra. The second is a decrease in proportion of 0–0 transition relative to 0–1 transition. The third is an obvious aggregation induced quenching in emission spectra. However it should be noticed that the concentration of carbazole unites was up to 2×10^5 ppm and inner filter effect was inevitable at such high concentration. Inner filter effect was previously considered as an error in fluorescence measurement, which resulted from absorption of the incident and/or emission light by the absorber in the detection system. [23, 24] Excitation inner filter effect could cause decrease in emission intensity and emission inner filter could cause a distortion of the shape of fluorescence spectrum. Therefore, it is necessary to work as much as possible with dilute solutions in order to exclude the influence of inner filter effect.

However, for a concentration induced aggregation process, inner filter effect is hardly avoided and corrected in high concentration solutions. For this reason, in this work, a solvent induced aggregation process was designed by dissolving N-vinyl carbazole in ethanol

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(solvent) first and precipitating in a mixed solvent of ethanol and water (non-solvent) with different water contents. Because the concentration of N-vinyl carbazole is as low as 1 ppm, the influence of inner filter effect could be well suppressed. By comparing the fluorescence spectra of solvent induced aggregation with that of concentration induced aggregation and corresponding polymer, it demonstrates that only the intensity ratio of 0–0 transition to 0-1transition is a qualified sensitive indicator for aggregation state of carbazole unites.

Experimental Section

Materials

N-vinyl carbazole and Poly(9-vinylcarbazole) (98%) were purchased from Aladdin Biological and Chemical Corp. (China). Ethanol (AR) was purchased from Chuandong Chemical Co., Ltd. All chemicals were used without further purification. Deionized water was supplied by a RO purifier (AHL-2001-P) from Aquapro.

Preparation of Carbazole Aggregates Dispersion

For concentration induced aggregation, N-vinyl carbazole was directly dissolved in ethanol at given concentrations. For solvent induced aggregation, N-vinyl carbazole was first dissolved in ethanol at the concentration of 1000 ppm. Then 10 μL N-vinyl carbazole ethanol solution was injected into 10 mL mixed solvents of ethanol and water with given water contents. Poly(9-vinylcarbazole) solution was prepared by dissolving in ethanol directly at the concentration of 1 ppm.

Characterization

All the excitation and emission spectra were recorded with a fluorescence spectrometer (Lengguang Tech, F97pro). Band pass used for recording fluorescence and fluorescence excitation spectra was 5 nm.

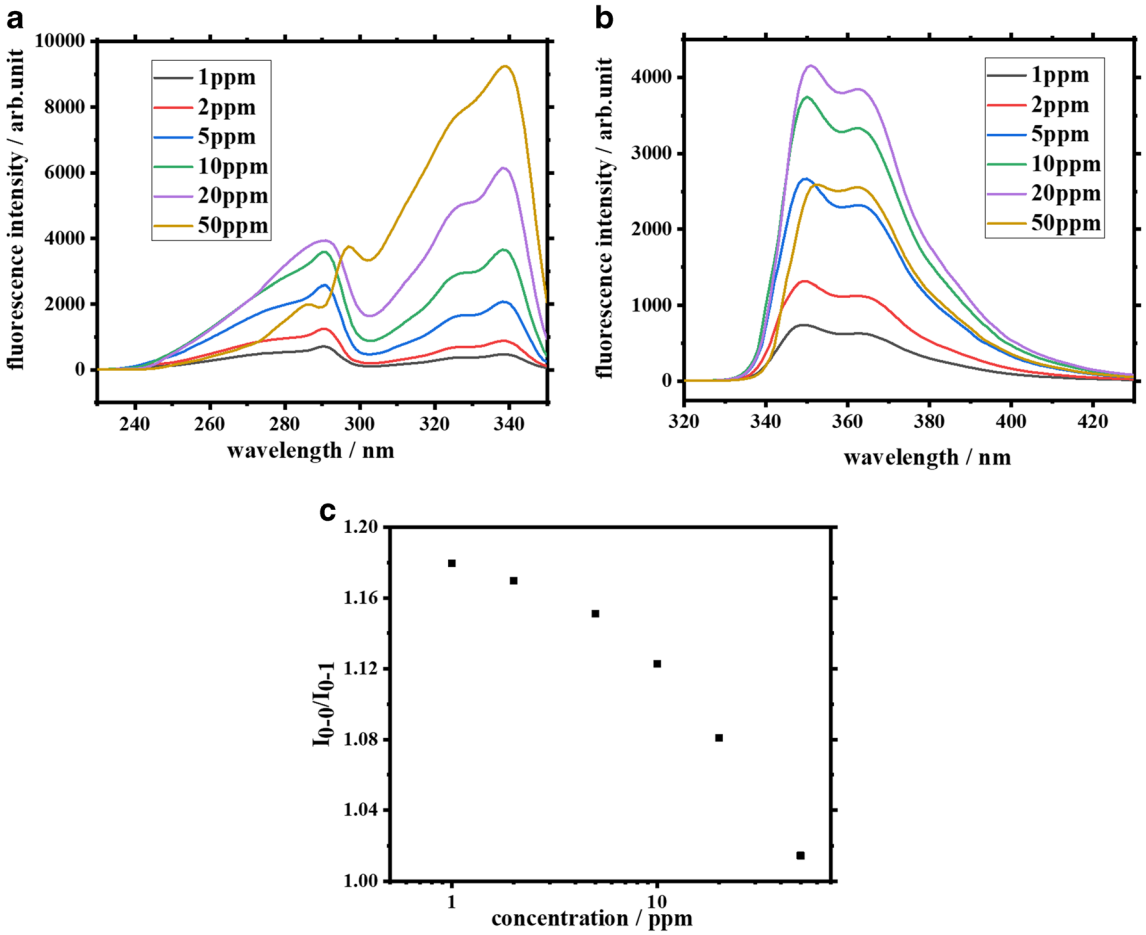

Fig. 1 Excitation spectra ($\lambda_{emission} = 363$ nm) (**a**) and emission spectra ($\lambda_{excitation} = 291$ nm) (**b**) of N-vinyl carbazole ethanol solution at different concentrations. **c** intensity ratios of 0–0 transition to 0–1 transition obtained from emission spectra at various concentrations

Results and Discussion

Concentration Induced Aggregation

As a control experiment, aggregation of carbazole units was first induced by increasing concentration. Figure 1 shows the excitation and emission spectra of N-vinyl carbazole ethanol solution with concentrations from 1 ppm to 50 ppm. There are two bands in excitation spectra (Fig. 1a). The band from 240 nm to 300 nm represents $S_0 \rightarrow S_2$ transition and the band from 300 nm to 350 nm corresponds to $S_0 \rightarrow S_1$ transition. Obviously there is a decrease in proportion of $S_0 \rightarrow S_2$ transition with increase in concentration. As to the emission spectra (Fig. 1b), there are two peaks which correspond to 0–0 transition at around 349 nm and 0–1 transition at around 363 nm. The total intensity increased slightly at first which is not proportional to the increase in concentration. In addition, when the concentration was higher than 20 ppm, the total emission intensity decreased with rising concentration indicating aggregation induced quenching. On the other hand, the intensity ratio of 0–0 transition to 0–1 transition decreases when concentration of carbazole units rises as shown in Fig. 1c. All these spectral features are in accordance with results in literature with respect to aggregation of carbazole unites. [22]

Solvent Induced Aggregation

Aside from aggregation, increase in concentration might cause inner filter effect. For this reason, it is necessary to design an aggregation process at a constant concentration. Mixing solvent and non-solvent should be an effective strategy to tuning solvent affinity, which was frequently employed to induce aggregation of substance. Here ethanol and water are solvent and non-solvent of N-vinyl carbazole respectively. Since the affinity between N-vinyl carbazole and water is extremely poor, it is difficult to dissolve N-vinyl carbazole in mixed solvents directly even with extremely low water contents. Thus, a nanoprecipitation process was adopted that dissolving

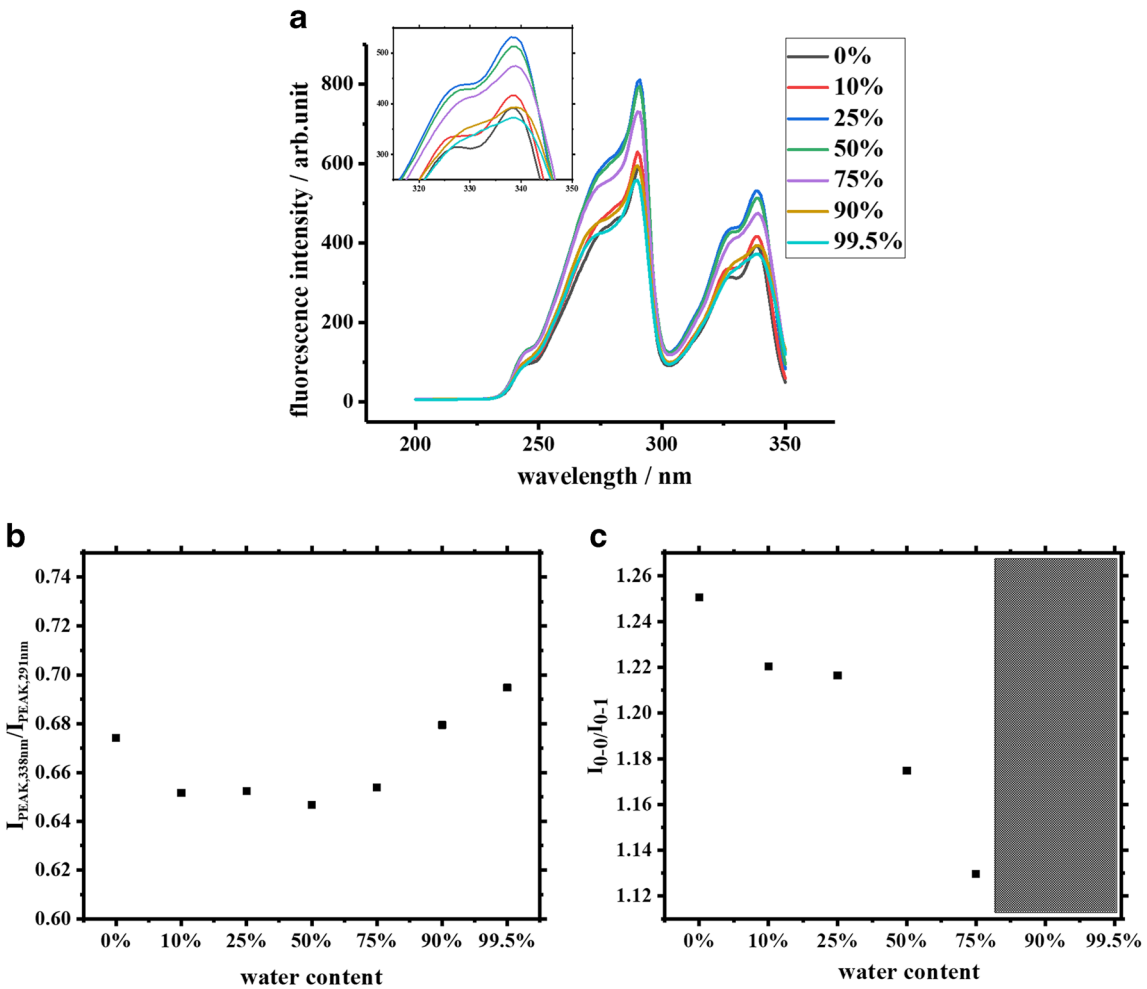

Fig. 2 **a** excitation spectra ($\lambda_{emission} = 363$ nm) of N-vinyl carbazole in mixed solvents of ethanol and water with different water contents (*w*/w). The inset is the magnification of $S_0 \rightarrow S_1$ transition. **b** intensity ratios of peak at 338 nm to peak at 291 nm. **c** intensity ratios of 0–0 transition to 0–1 transition. (c = 1 ppm)

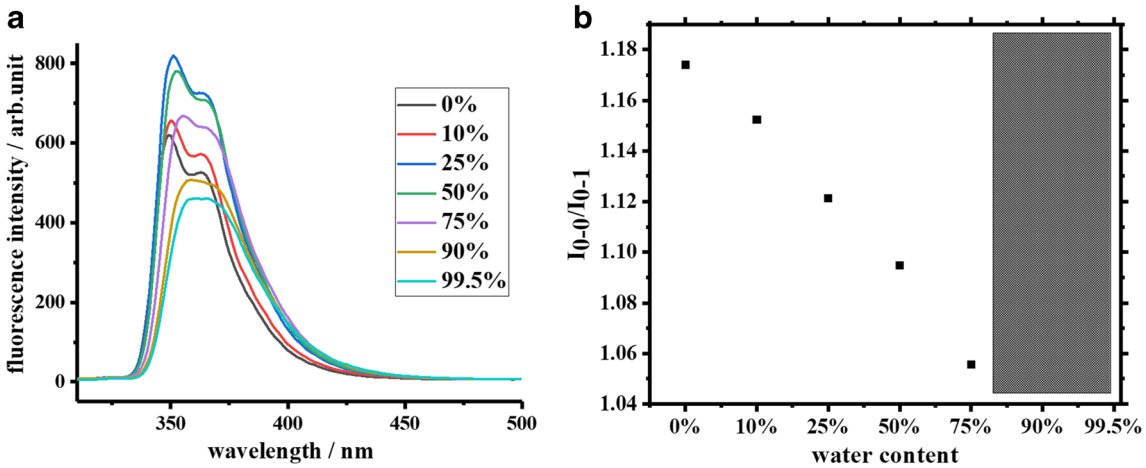

Fig. 3 **a** emission spectra ($\lambda_{excitation}$ = 291 nm) of N-vinyl carbazole in mixed solvents of ethanol and water with different water contents (*w*/w). **b** intensity ratios of 0–0 transition to 0–1 transition. (c = 1 ppm)

N-vinyl carbazole in ethanol at first and then precipitating in mixed solvent of ethanol and water. With the aid of nanoprecipitation, N-vinyl carbazole can be dispersed in ethanol-water solution and keep stable for several days at extremely low concentration. On the other hand, it is very easy to control the aggregation of N-vinyl carbazole by mixing ethanol and water at different compositions. More and larger aggregates formed at higher water contents.

Figure 2a shows the excitation spectra at different water contents. Different from concentration induced aggregation, there is no such an obvious decrease in proportion of $S_0 \rightarrow S_2$ transition upon increase in water contents (Fig. 1a). It can be found that the intensity ratios of emission peaks at 338 nm to 291 nm keep constant only slightly changing around 0.67 (Fig. 2b). As to $S_0 \rightarrow S_1$ transition, there are two peaks at about 329 nm and 338 nm, which should correspond to 0–1 transition and 0–0 transition respectively in excitation process. The intensity ratio of 0–0 transition to 0–1 transition decreases from 1.25 to 1.13 upon increase in water contents as shown in Fig. 2c. When water contents are beyond 0.75, it is difficult to observe the fine structure and two vibronic peaks merge into a broad peak in excitation spectra. According to the analysis of excitation spectra, it might be concluded that intensity ratio of 0–0 transition to 0–1 transition should be an indicator to aggregation of carbazole unites. While the decrease in proportion of $S_0 \rightarrow S_2$ transition may result from inner filter effect at high concentration.

From emission spectra at different water contents in Fig. 3a, the emission intensity increases when water contents rise from 0 to 25%. Reaching a maximum, the emission intensity decreases when water contents rise from 25% to 99.5%. According to the comparison of emission spectra in pure ethanol and nearly pure water, in other words the molecular state and aggregated state, there is only a slight decrease in emission intensity. Intensive quenching in concentration induced aggregation has not been observed. In contrast, the emission intensity in pure water is even higher in the range from 380 nm to 400 nm. The emission in such region was ascribed to the excimer in literatures. [25, 26] Thus so-called aggregation induced quenching in previous study should be ascribed to inner filter effect at high concentration. [22] Concerning the fine structures of emission spectra, there are two emission peaks at about 350 nm and 363 nm corresponding to 0–0 transition and 0–1 transition respectively in emission process. A red shift of 0–0 transition peak is presented and the relative intensity of 0–0 transition decreases upon increase in water contents (Fig. 3a). The intensity ratio of 0–0 transition to 0–1 transition decreases from 1.17 to 1.06 monotonously when water contents increase from 0 to 75% (Fig. 3b). Similarly, fine structure of emission spectra disappears and a broader peak is observed When water contents are beyond 0.75.

Based on the spectral analysis of excitation and emission spectra, it is clearly shown that intensity ratio of 0–0 transition

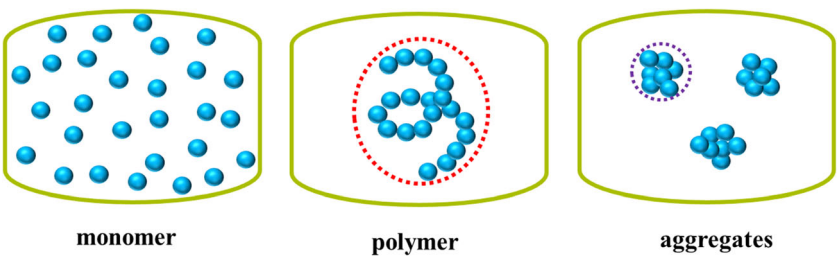

Fig. 4 Schematic illustration of N-vinyl carbazole in different aggregation states

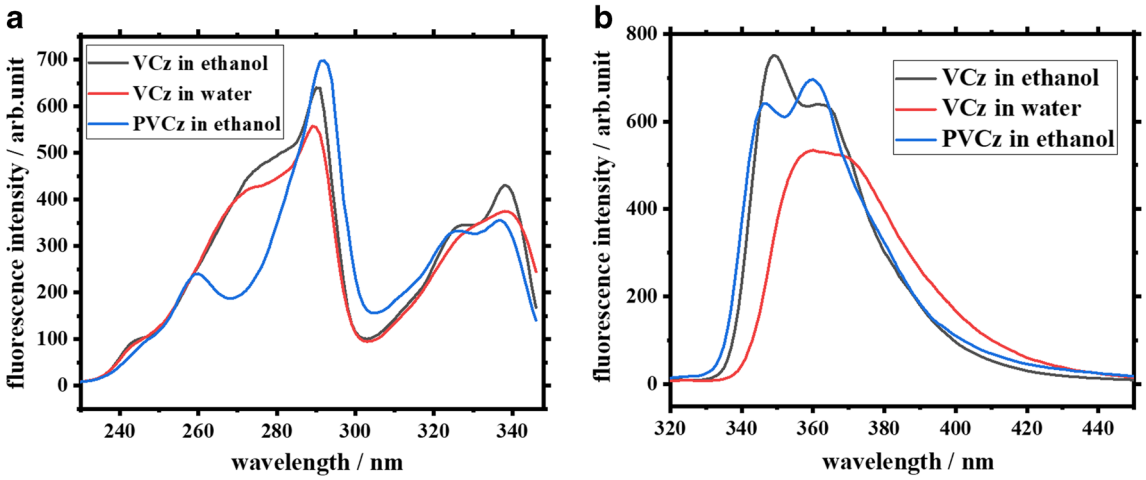

Fig. 5 Excitation ($\lambda_{emission} = 363$ nm) (**a**) and emission ($\lambda_{excitation} = 291$ nm) (**b**) spectra of carbazole unites in different aggregation states. VCz and PVCz are short for N-vinyl carbazole and r poly N-vinyl carbazole respectively

to 0–1 transition is a sensitive indicator for aggregation of carbazole unites. The proportion of 0–0 transition decreases upon aggregation. According to the comparison of spectra in concentration induced aggregation and solvent induced aggregation, we consider that aggregation induced quenching and decrease in proportion of $S_0 \rightarrow S_2$ transition upon aggregation should result from inner filter effect.

Comparison of Monomer, Aggregates and Polymers

In order to further confirm these spectral features, comparison of N-vinyl carbazole between three different aggregation states has been performed: monomer (single molecule), polymer and aggregates. Because monomers and polymers of N-vinyl carbazole can be dissolved in ethanol at low concentration, thus monomer and polymer states were obtained by dissolving it directly in ethanol. The aggregates were obtained by dispersing N-vinyl carbazole in water with the aid of nanoprecipitation process as described in "Experimental" section. According to a schematic illustration in Fig. 4, even the mole concentrations of carbazole unites are the same, local concentrations of carbazole unites in three states are totally different, which are more suitable to describe different aggregation states. Local concentration in aggregates is highest, higher than that in polymer and the local concentration in monomer is lowest. From the excitation spectra in Fig. 5a, it can be found that the peak at about 270 nm seems to disappear in excitation spectrum of polymer. This peak should correspond to double bonds in N-vinyl carbazole which vanished during polymerization. As to $S_0 \rightarrow S_1$ transition (band from 300 nm to 350 nm), there are distinct two peaks in the excitation spectra of either monomer or polymer. The intensity ratios of 0–0 transition (338 nm) to 0–1 transition (329 nm) are 1.25 and 1.07 for monomer state and polymer state respectively. In the meantime, only a broad peak without fine structure exists in that of aggregates. On the other hand, the comparison of emission spectra (Fig. 5b) gives similar results. The intensity ratios of 0–0 transition (around 348 nm) to 0–1 (363 nm) transition are 1.17 and 0.92 for monomer state and polymer state respectively. Similarly, only a broad emission peak without distinguishable vibronic structures displays in the emission spectrum of aggregates. These results also indicate that lower proportion of 0–0 transition at higher local concentration, which is in consistent with the conclusion drawn from solvent induced aggregation experiments.

Conclusion

Aggregation processes of N-vinyl carbazole resulting from increase in concentration or addition of a non-solvent were studied by fluorescence spectroscopy. From both excitation and emission spectra, it can be found that the proportion of 0–0 transition relative to 0–1 transition decreases upon aggregation. In addition, decrease in proportion of $S_0 \rightarrow S_2$ transition and aggregation induced quenching reported in previous study were ascribed to inner filter effect resulting from high concentration. Investigation into different aggregation states of N-vinyl carbazole: monomer, polymer and aggregates, furtherly supports that higher local concentration induced decrease in relative intensity of 0–0 transition. The intensity ratio of 0–0 transition to 0–1 transition should be a special spectral feature in aggregation of carbazole unites, which have the potential to become a sensitive indicator in application of probes and sensors based on carbazole unites.

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