Abnormal Fluorescence Behavior of Pyrene Functionalized Chitosan Films to Some Quenchers

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Investigation of the fluorescence behavior of a pyrene functionalized chitosan film (PSC-CS) to some quenchers, including KI, CH_3NO_2 and $Cu(NO_3)_2$, has revealed that the monomer emission from the PSC-CS film increased rather than decreased, whereas the excimer emission decreased with the increase of time of duration after addition of KI or $Cu(NO_3)_2$. Both the increasing and decreasing processes could last as long as 20 min to 40 min. Unlike that observed in the system containing KI or $Cu(NO_3)_2$, for CH_3NO_2 system, both the monomer emission and the excimer emission increased with introduction of the quencher, but the excimer emission only started to increase after an hour-long induction period. The position of the excimer emission of the PSC-CS film did not change very much with addition of KI or $Cu(NO_3)_2$. However, addition of CH_3NO_2 made the excimer emission blue shifted for at least 20 nm indicating formation of distorted excimers or partially overlapped excimers. The fluorescence response of the film to external additions is sensitive but is low in selectivity. This property may make the film use as a novel sensing material for monitoring the purity of water.

Keywords chitosan, pyrene, fluorescence quenching, water purity

Introduction

Functional films are intensively studied due to their wide and potential applications in separation, biomedicine, sensing and intelligent systems.[1] In the current series of work, pyrene (**Py**) was doped into or chemically immobilized onto chitosan film in order to prepare, initially, a chemical sensor for solvent polarity measurement. This is because the steady state fluorescence emission spectrum of pyrene is composed of five vibronic bands, and the ratio of the intensity of band 3 to band 1 is a measure of the polarity of the medium.[2-4]

We have recently described the preparation and fundamental fluorescence properties of the **Py** functionalized chitosan (**CS**) film.[5,6] The sensing properties of the film prepared by simple embedding of **Py** is quite good, but the service life of the film is short due to leaking of the active species. The sensitivity of **Py** chemically functionalized **CS** film (**PSC-CS**) is not as good as that expected. But a quite surprising result was observed. The monomer emission of the film was weak both at dry and wet states if super-pure water was used to wet the film. However, upon addition of salts or replacing the super-pure water with tap water the monomer emission increased dramatically whereas the change in excimer emission was hardly observed. More surprisingly, the monomer emission returned to the original intensity at once when the film was washed with super-pure water for several times, and furthermore the process could be repeated for several times. Here another abnormal behavior of the **PSC-CS** film to some fluorescence quenchers, including KI, $Cu(NO_3)_2$, and CH_3NO_2, is reported.

Materials and methods

Py was purchased from Acros (96%) and re-crystallized from ethanol for more than three times before use. **CS** was a product of our laboratory.[7-10] Its viscosity average molecular mass was 1.9×10^5. The degree of de-acetylation was nearly 100%. Water was purified by de-ionization and double distillation before use. KI, $Cu(NO_3)_2$, CH_3NO_2 and other chemicals were obtained from Xi'an Chemicals and of at least analytical grade.

CS films were prepared in acetic acid medium using formaldehyde as a cross-linker.[5,6] Pyrene-sulfonyl chloride (**PSC**), the precursor for the immobilization of **Py**, was prepared with sodium pyrene-sulfonate (PSA) as an intermediate according to a literature method.[11] The general synthetic route for the preparation of the **PSC-CS** film is shown in Scheme 1. The details of the preparation and the fundamental physical properties of the film have been reported earlier.[6]

Fluorescence measurements were performed at room temperature on a Perkin-Elmer LS 50B luminescence spectrometer. The **PSC-CS** film (0.7 cm × 3.0 cm) was inserted into a quartz cell with its active surface facing the excitation light source. The cell was fixed in the solid sample holder of the instrument. The position of the film was kept constant during each set of measurements. For monitoring the fluorescence

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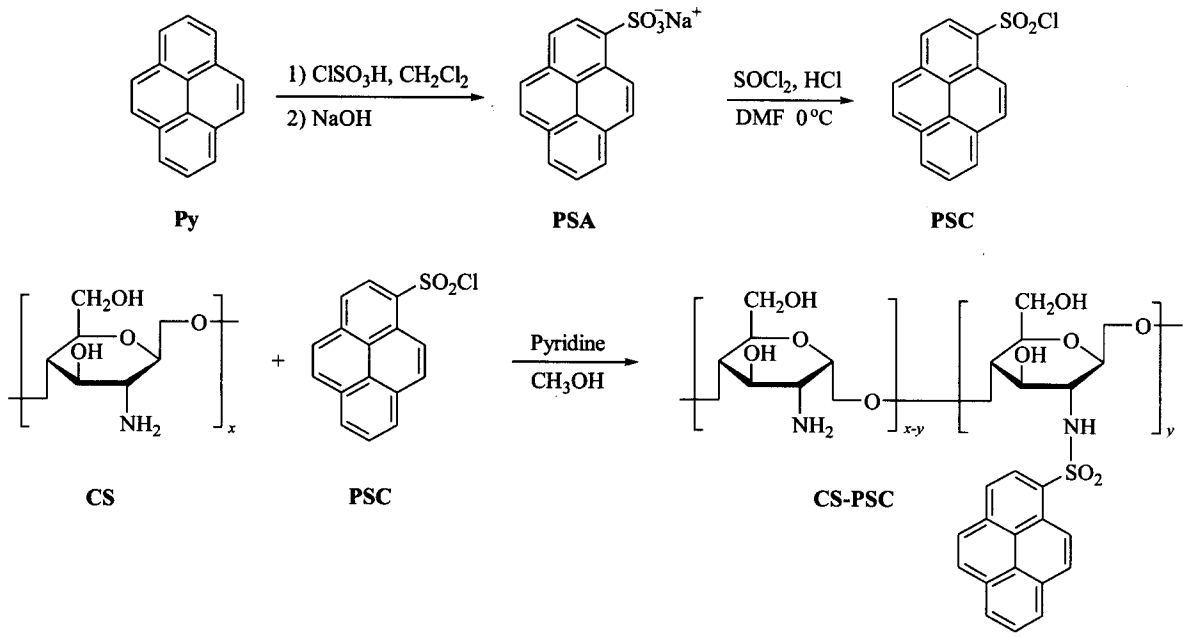
Scheme 1



change with time, an automatic measurement with preset time interval method was employed. The emission was deducted to 1% of the real intensity to allow long time monitoring.

Results and discussion

The fluorescence excitation and emission spectra of the **PSC-CS** film both at dry and wet states are shown in Fig. 1. It is revealed that the emission spectrum of the film is mainly composed of two parts. The first part (360 nm—*ca*. 440 nm) is characterized by a few sharp bands, which have been assigned to the monomer emission from **Py** in immobilized state.[12,13] The second part is that from 440 nm to the far end of the emission spectrum. This part is a simple and broad structureless band, which is characteristics of an excimer emission.[14-17] Clearly, wetting has little effect upon the fundamental fluorescence properties of the film. Far from that expected, however, introduction one of the normally used efficient, nonspecific fluorescence quenchers including KI, $Cu(NO_3)_2$ and CH_3NO_2 did not quench the monomer fluorescence from 360 nm to *ca*. 420 nm as reported later.

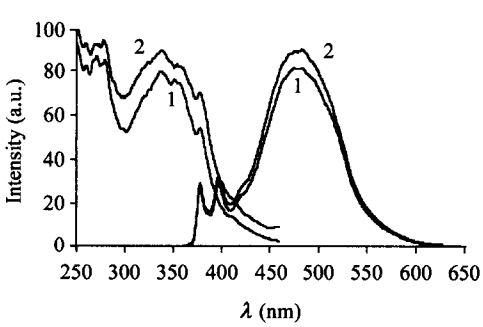


Fig. 1 Fluorescence excitation and emission spectra of the **PSC-CS** film at dry (1) and wet (2) states (λ_{ex} = 340 nm, λ_{em} = 479 nm).

Fig. 2 shows a few fluorescence emission spectra of the **PSC-CS** film recorded at different time intervals after addition of $Cu(NO_3)_2$. It can be revealed that in the presence of $Cu(NO_3)_2$ the monomer emission increased dramatically and the excimer emission decreased with the increase of time. This phenomenon can last as long as 40 min. When KI was used as a quencher, the result was similar with that observed in the system containing $Cu(NO_3)_2$. But at this case, both the increasing and decreasing were not as significant as those observed in the former system. The observation may be understood by considering that **Py** on the **PSC-CS** film might have aggregated greatly. Introduction of the two quenchers might make the aggregates dissociate, and thereby increase the content of isolated monomer. In this way, increase in monomer emission should be accompanied by decrease in excimer emission supposing fluorescence quantum yield of the monomer and the excimer did not change during the process. It is known that both $Cu(NO_3)_2$ and KI are normally used quenchers, in particular, the bimolecular quenching constant of $Cu(NO_3)_2$ can be more than five orders of magnitude larger than that of a diffusion controlled quenching rate expected for homogeneously distributed systems.[18-21] But, why did addition of the quenchers,

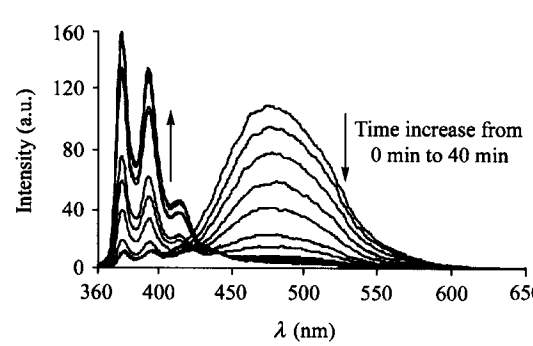


Fig. 2 Fluorescence emission spectra of the **PSC-CS** film immersed in the solution of $Cu(NO_3)_2$ (0.1 mol/L) measured every 5 min (λ_{ex} = 340 nm).

in particular $Cu(NO_3)_2$, not show any quenching effect for the monomer emission of the **PSC-CS** film? This may be understood from the following considerations. As discussed earlier, both $Cu(NO_3)_2$ and KI did function as fluorescence quenchers as shown by decrease in the excimer emission shown in Fig. 2, which had not been found in other system containing $NaNO_3$, $Ca(NO_3)_2$ or $Al(NO_3)_3$.[6] It is to be noted, however, that the two quenchers also behaved like salts. This is because the monomer emission of the **PSC-CS** film increases along with the addition of one of the salts, including $NaNO_3$, $Ca(NO_3)_2$ or $Al(NO_3)_3$,[6] a similar observation with that obtained from the systems containing KI or $Cu(NO_3)_2$. Clearly, for the current study, $Cu(NO_3)_2$ and KI function both as quenchers and sensitizers, and the observation that addition of one of the two quenchers sensitized the monomer fluorescence and quenched the excimer fluorescence is a net result of the two effects.

The observation is even surprising for the system where CH_3NO_2 was used as a quencher. At this case, both monomer and excimer emission increased with extension (*cf.* Fig. 3), a typical sensitizing effect. The nature of the sensitizing is not clear at this moment. But by comparison of the emissions shown in Fig. 2 and those shown in Fig. 3, it can be recognized that the maximum of the excimer emission from the system containing CH_3NO_2 is blue shifted for more than 20 nm indicating existence of distorted excimer or partially overlapped excimer structures.

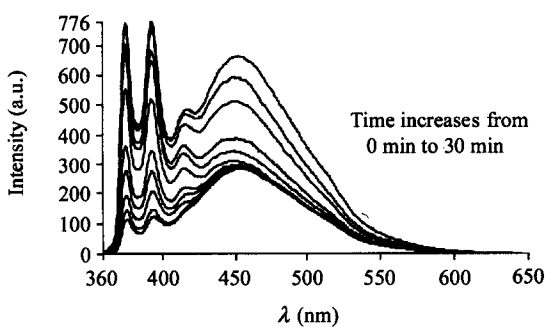


Fig. 3 Fluorescence emission spectra of the **PSC-CS** film immersed in the solution of CH_3NO_2(0.1 mol/L) measured every 5 min (λ_{ex} = 340 nm).

It is known that excimer formation is due to the interaction of an excited **Py** species with a **Py** molecule in ground state.[22] The interaction is both orientation and distance dependent. The required inter-planar distance for a perfect sandwich-like structure is about 0.35 nm[23] For the current study, excimer formation on the surface of the **PSC-CS** film was detected by the presence of a broad structureless band centered at 480 nm (*cf.* Fig. 1). Changes in excimer structure must be originated from the changes in the distribution of the immobilized **Py** on the surface of the matrix of the film. The redistribution can only be attributed to the specific interaction between the quencher, CH_3NO_2 and the matrix, **CS**. Addition of CH_3NO_2 to the system containing **PSC-CS** film might increase the swelling degree of the film, and thereby increase the distance between the neighboring **Py** species. As a result, the content of **Py** in isolated state may increase and the excited dimer (excimer) may be distorted, as demonstrated in the Schematic diagram shown in Fig. 4. Decrease in the density of **Py** species on the film due to swelling may be favorable for the increase in fluorescence intensity due to reduction in self-quenching. Unlike CH_3NO_2, addition of KI or $Cu(NO_3)_2$ has little effect upon the structure of the excimer as evidenced by the un-change of the position of the excimer emission (*cf.* Fig. 2).

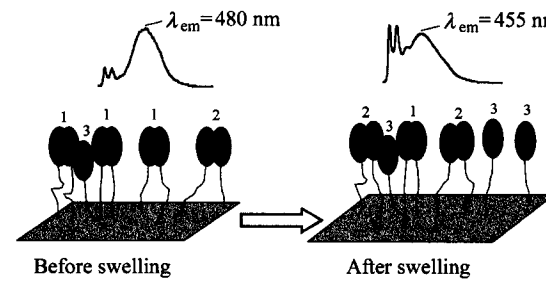


Fig. 4 Illustration of the blue shift of the excimer emission of the **PSC-CS** film with addition of CH_3NO_2 (1: perfect excimer, 2: distorted excimer, 3: **Py** in isolated state).

Fig. 5 (a) and (b) depict the time dependence of the monomer and excimer emission of the **PSC-CS** film immersed in various mediums. With reference to the figures, it can be revealed that for the system where super-pure water was used as a medium both the monomer emission and the excimer emission did not change with time. For systems containing KI or $Cu(NO_3)_2$ the increase in monomer emission and decrease

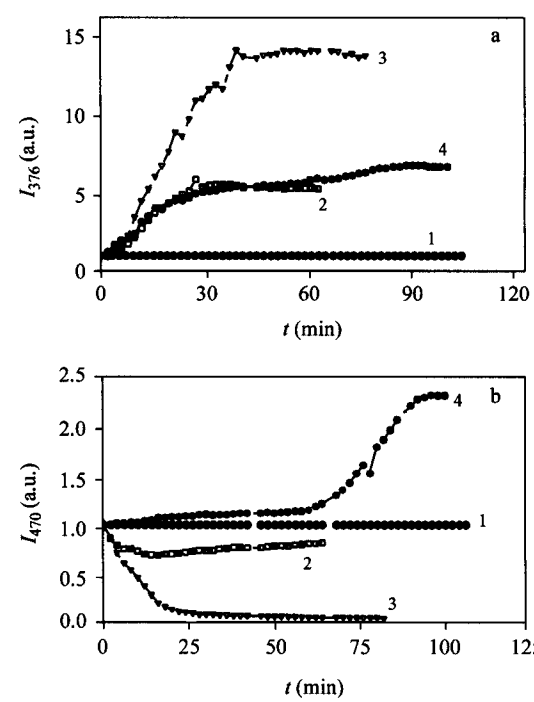


Fig. 5 Time dependence of the monomer emission (a) and the excimer emission (b) of the **PSC-CS** film in (1) super-pure water, (2) KI, (3) $Cu(NO_3)_2$ and (4) CH_3NO_2 (concentration 0.1 mol/L, λ_{ex} = 340 nm).

in excimer emission occurred at once after addition of the salts. For the system where CH_3NO_2 was introduced, the increase in the monomer emission happened, as expected, immediately after introduction of the quencher. However, the excimer emission only started to increase after a long induction period (*cf.* Fig. 5(b)). It may not be easy to explain why the monomer emission and excimer emission did not change simultaneously. But it is believed, again, that the interaction between the quencher and the matrix of the **PSC-CS** film plays crucial role in the process. To verify the functions of the film matrix for the abnormal fluorescence behavior of the **PSC-CS** film, **Py** was successfully immobilized onto a surface modified quartz plate, which was chosen as an inert matrix for another functional film to be prepared. The fluorescence behavior and sensing properties of the new **Py** functionalized film was under examination.

As far as the time dependence of the ratio of the excimer emission to the monomer emission was concerned, CH_3NO_2 behaved similarly with those of other two quenchers studied (*cf.* Fig. 6). Upon addition one of the quenchers, the ratio decreases significantly with extension. The decrease slows down about ten minutes later, and actually keeps constant another five minutes later except an obvious increase in the ratio for the system containing CH_3NO_2 an hour later. Clearly, the sharp decrease in the ratio at the beginning after addition one of the quenchers is a result of the fact that increase in excimer emission is much slower than that in monomer emission. The situation where the ratio keeps constant may be either a result of simultaneously increase of the two emissions with the ratio keeping constant or both emission does not change with time (*cf.* Fig. 5). For the system containing CH_3NO_2, the ratio starts to increase an hour later. The reason for the increase is obvious by referencing to Fig. 5(a) and (b), where the excimer emission starts to increase after a long period of induction.

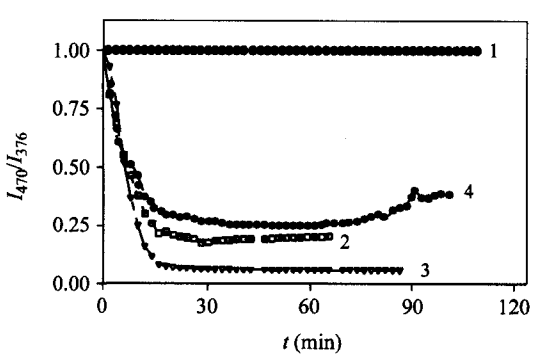


Fig. 6 Time dependence of the ratio of the excimer emission to monomer emission of the **PSC-CS** film in (1) super-pure water, (2) KI, (3) $Cu(NO_3)_2$ and (4) CH_3NO_2 (concentration 0.1 mol/L, λ_{ex} = 340 nm).

Similar time dependent pattern of the ratio for the systems containing various fluorescence quenchers may be a reflection of the fact that the fluorescence response of the **PSC-CS** film to external additions is sensitive but is low in selectivity. This property may make the film useful as a novel fluorescence sensor for monitoring water purity.

Conclusion

It has been demonstrated that immobilization of **Py** onto **CS** film yielded a new fluorescence film. The responsive property of the fluorescence active film to the addition of one of the common quenchers, including KI, $Cu(NO_3)_2$ and CH_3NO_2 is not the traditional decrease, but increase in the monomer emission. Considering the responsive property of the film to other external additions including surfactants, salts and tap water,[6] it may be concluded that addition of any of external "impurities" makes the fluorescence intensity of the film increase. The low selectivity of the film to external additives may make it a novel sensing material for monitoring the purity of water.

References

1 Akelah, A.; Moet, A. *Functionalized Polymers and Their Applications*, Chapman and Hall, Boston, **1990**.
2 Nakajima, A. *Bull. Chem. Soc. Jpn.* **1971**, *B44*, 3272.
3 Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1977**, *99*, 2039.
4 Dong, D. C.; Winnik, M. A. *Can. J. Chem.* **1984**, *62*, 2561.
5 Ning, G. H.; Lu, J. R.; Fang, Y; Hu, D. D. *Chem. J. Chin. Univ.* **2000**, *21*, 1196 (in Chinese).
6 Fang, Y.; Ning, G. H.; Hu, D. D.; Lu, J. R. *J. Photochem. Photobiol.* **2000**, *135*, 141.
7 Fang, Y.; Hu, D. D. *Chin. J. Polym. Sci.* **1999**, *17*, 551 (in Chinese).
8 Wang, M. Z.; Qiang, J. C.; Fang, Y; Hu, D. D.; Cui, Y. L.; Fu, X. G. *J. Polym. Sci. Part A: Polym. Chem.* **2000**, *38*, 474.
9 Fang, Y.; Liu, S. X.; Hu, D. D.; Cui, Y. L.; Xue, M. *Polym. Bull.* **1999**, *43*, 387.
10 Qiang, J. C.; Wang, M. Z.; Fang, Y; Hu, D. D.; Cui, Y. L.; Wang, Y. Q. *Acta Chim Sinica* **2000**, *58*, 627 (in Chinese).
11 Ezzell, S. A.; McCormick, C. L. *ACS Symposium*, Series 467, ACS, Washington DC, **1991**.
12 Chu, D. Y.; Thomas, J. K. *Macromolecules* **1984**, *17*, 2142.
13 Olea, A. F.; Thomas, J. K. *Macromolecules* **1989**, *22*, 1165.
14 Ezzell, S. A.; McCormick, C. L. *Macromolecules* **1992**, *25*, 1881.
15 Ezzell, S. A.; Hole, C. H.; Creed, D.; McCormick, C. L. *Macromolecules* **1992**, *25*, 1887.
16 Kramer, M. C.; Steger, J. R.; Hu, Y. X.; McCormick, C. L. *Macromolecules* **1996**, *29*, 1992.
17 Char, K.; Gast, A. P.; Frank, C. W. *Langmuir* **1988**, *4*, 989.
18 Delair, J. A.; Rodgers, M. A. J.; Webber, S. E. *J. Phys. Chem.* **1984**, *88*, 6219.
19 Arora, K. S.; Turro, N. J. *J. Polym. Sci., Part A: Polym. Chem.* **1987**, *25*, 259.
20 Morrison, M. E.; Dorfman, R. C.; Clendening, W. D.; Kiserow, D. J.; Rossky, P. J.; Webber, S. E. *J. Phys. Chem.* **1994**, *98*, 5534.
21 Morrison, M. E.; Dorfman, R. C.; Webber, S. E. *J. Phys. Chem.* **1996**, *100*, 15187.
22 Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd Ed., Kluwer Academic/Plenum Publishers, New York, **1999**.
23 Kayanasundaram, K. *Photochemistry in Microheterogeneous Systems*, Academic Press, New York, **1987**.

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