Monitoring the Aggregation of Dansyl Chloride in Acetone through Fluorescence Measurements

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The aggregation of dansyl chloride (DNS-Cl) in acetone has been studied in detail by steady-state fluorescence techniques. It has been demonstrated that DNS-Cl is stable in acetone during purification and aggregation study processes. The aggregates are not solvolyzed in acetone, and do not take part in any chemical reactions either. It has been found that DNS-Cl tends to aggregate even when its concentration is much lower than its solubility in acetone. The aggregation is reversible, and both the aggregation and the deaggregation are very slow processes. Introduction of SDS has a positive effect upon the formation and stabilization of the aggregates.

Keywords dansyl chloride, acetone, aggregation, fluorescence techniques

Introduction

Dansyl chloride (DNS-Cl) is widely used in labeling proteins,[1,2] carbohydrates[3,4] and synthetic polymers,[5-7] for the studies of their physical behaviors and the determination of their quantities in aqueous phase, which is due to the fact that its emission spectrum is highly sensitive to solvent polarity.[8] Based upon the properties of the fluorophore, it has been tried to label the DNS-Cl onto chitosan films and aminated quartz plates via the sulfonation of the amino groups on the surface of the substrates in order to prepare, initially, a chemical sensor for solvent polarity measurement.[9,10] The solvent used in the immobilization was acetone. It was found that DNS-Cl has a strong tendency to aggregate in acetone even though the concentration is very low. As might be expected, the aggregation of the fluorophore results in an inhomogeneous labeling of the fluorophore on the film or on the plate surface, and thereby affects the sensing properties of the materials. To the best of our knowledge, there has been not any report on the aggregation of DNS-Cl in acetone. Therefore, we investigate the aggregation in detail.

Experimental

Materials and instruments

Purified DNS-Cl was obtained by extracting the reagent of DNS-Cl(Aldrich) with dry acetone in a Soxhlet's extractor. The profile of the IR spectrum of the purified DNS-Cl is basically the same as that of the standard spectrum of the reagent. Elemental analysis results (%): C 57.2, H 5.4, N 5.3, are very close to the theoretical values (%): C 57.3, H 5.2, N 5.6. Acetone used in the present study was of spectral grade, and was dried completely with molecular sieve before use.

Sodium dodecyl sulfate (SDS) of super-pure grade is a product of BDH . It was dried with anhydrous $CaCl_2$ for several days before use.

Pressed KBr disk was used for transmission infrared spectroscopy measurement, which was performed on a Equinox 55 FTIR spectrometer.

All fluorescence measurements were conducted on a Perkin-Elmer LS 50B luminescence spectrometer. For monitoring the changes in the fluorescence spectrum, an

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

Preparation of the saturated solution of DNS-Cl *in acetone*

An excess amount of pure DNS-Cl was put in a reagent bottle with 200 mL of dry acetone. The bottle was sealed and shaken at 25 ℃ for more than 1000 min. About 3 mL of the supernatant was carefully withdrawn from the top of the bottle, and kept in a small tube. 35 samples were collected in this way. The fluorescence intensity of each sample was measured by taking 337 nm and 520 nm as excitation and analysis wavelengths, respectively. It was found that the fluorescence intensity of the sample tends to be constant after 180 min equilibration, indicating that an solubilization equilibrium was reached. Based upon this experiment, the saturated DNS-Cl solution was prepared by filtration of the above suspension with glass sand filter after 200 min equilibration. The saturated solution prepared in this way was kept in a sealed brown bottle at room temperature and used as a stock solution during the experiment.

Solubility of DNS-Cl *in acetone*

A weighing bottle of 10 mL volume was dried at 50 ℃ for several hours till a constant weight (w_1), and then 10.00 mL of the saturated DNS-Cl solution (V) was transferred into it. The bottle was left in a dust free place at room temperature until the solvent was evaporated completely, and then the bottle was dried in an oven of a temperature of 50 ℃ (note: the m.p. of DNS-Cl is 72—74 ℃) till a constant weight (w_2). The solubility (S) of DNS-Cl in acetone was calculated by using the following equation:

$$S = \frac{w_2 - w_1}{V} \times 1000 \qquad (1)$$

The determination was repeated for three times and found that the solubility of DNS-Cl in acetone at 25 ℃ is 702 mg/L.

Concentration dependence of the fluorescence emission spectrum of DNS-Cl *in acetone*

A series of DNS-Cl solutions of different concentrations were prepared by dilution of the saturated DNS-Cl solution with dry acetone. The solutions were shaken for more than 2 h to ensure equilibration before spectroscopic measurement. The results are shown in Fig. 1.

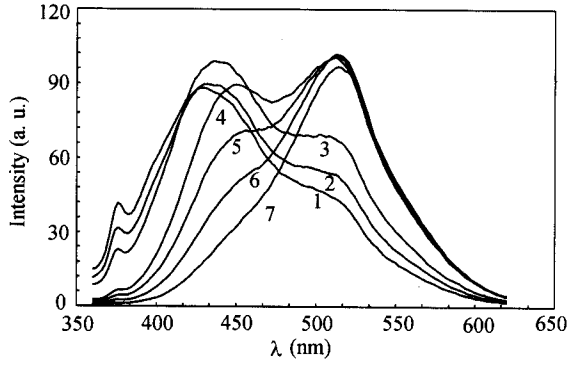

Fig. 1 Concentration dependence of the fluorescence emission spectrum of DNS-Cl in acetone (1: 5.2 mg/L; 2: 9.3 mg/L; 3: 15.6 mg/L; 4: 46.7 mg/L; 5: 56.0 mg/L; 6: 70.0 mg/L; 7: 105 mg/L).

Effect of SDS *upon the aggregation of* DNS-Cl

To 3 portions (0.6 mL each) of the saturated solution of DNS-Cl in acetone, (1) 4.4 mL of dry acetone; (2) 3.8 mL of dry acetone + 0.6 mL of saturated solution of SDS in acetone; (3) 4.4 mL of saturated solution of SDS in acetone was added, respectively. The mixtures were shaken for 2 min, and then the fluorescence spectrum of each sample was measured every hour. The fluorescence intensities at 450 nm and 513 nm, denoted as I_{450} and I_{513}, respectively, were recorded. The ratio of I_{450}/I_{513} was plotted against time (Fig. 4).

Results and discussion

Aggregation of DNS-Cl *in acetone*

Fig. 1 shows the dependence of the profile of the fluorescence emission spectrum of DNS-Cl in acetone upon the concentration of the solute at equilibrium state. With reference to the figure, it can be noted that the characteristic of the monomer emission decreases and that of the excimer emission increases with increasing the concentration of DNS-Cl. Further examination of the figure reveals that the excimer emission is becoming obvious when the concentration of the fluorophore is only 5.2 mg/L, indicating that DNS-Cl may have a strong tendency to

aggregate. The reason for the aggregation may be attributed to the presence of the groups in DNS-Cl which are poorly compatible with the solvent, acetone. To verify if there is any significant amount of ground state associates in the solution, the excitation spectrum of DNS-Cl with a concentration of 56 mg/L was measured at the analysis wavelengths of 420 nm and 520 nm, respectively. The results are shown in Fig. 2. It is obvious that the excitation spectrum corresponding to an analysis wavelength of 520 nm is significantly red shifted compared with the one analysed at 420 nm. Based upon this observation, it may be concluded that the solubilized DNS-Cl in acetone exists not only in solvated monomer state, but also in aggregated state.

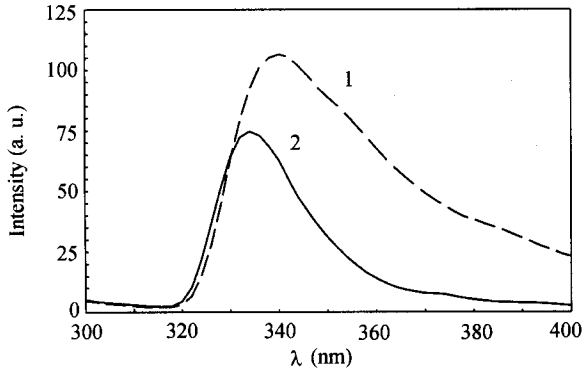

Fig. 2 Analysis wavelength dependence of the excitation spectrum of DNS-Cl (1: λ_{em} = 520 nm; 2: λ_{em} = 420 nm).

It is to be noted that although the critical aggregation concentration ([CAgC]) of DNS-Cl in acetone could not be determined accurately, the concentration must be much lower than the solubility of DNS-Cl in the solvent. This result is in support of Jiang's statement that [CAgC] and solubility are two different concepts in nature.[11] The aggregation behavior of DNS-Cl in acetone may be explained in consideration of its molecular structure. In the gas state, DNS-Cl should adopt the structure shown in Fig. 3. The corresponding dipole moment in this structure is 7.07 D. Considering that the dipole moment of acetone in gas state is only 2.88 D and the dielectric enrichment effect,[12] it is not surprising that DNS-Cl tends to aggregate in acetone. Actually, the aggregation of organic molecules in aqueous phase and mixtures of aqueous and organic solvent have been reported widely.[13,14] However, studies on the aggregation of organic molecules in pure organic solvent are limited.

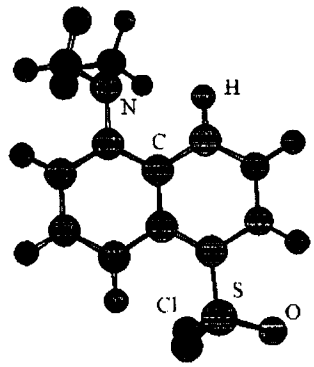

Fig. 3 3D structure of DNS-Cl in gas state (Note: the substitute group above the naphthylene ring is dimethylamino group, and the one bellow the ring is sulfonyl group).

Transformation between monomer state and association state of DNS-Cl

Change of the fluorescence emission spectrum of newly prepared DNS-Cl solution in acetone (56 mg/L) was automatically monitored with time intervals of 10 min. The results are shown in Fig. 4. With reference to the figure, it is revealed that the spectrum of the system at the beginning of the experiment is dominated by the excimer emission around 515 nm, indicating that the fluorophore exists mainly in aggregated state. With the elongation of time, the monomer emission around 440 nm increases faster than the excimer emission. Clearly, building a new equilibrium takes more than 7 h. This result shows that the aggregation of DNS-Cl and the dissociation of the aggregates in acetone are reversible and the transformation between the monomer state and the association state is very slow. Further examination of the figure reveals that the whole emission increases slowly with the elongation of time. It may be explained at the beginning of the experiment, DNS-Cl mainly exists in aggregated state and the solution was not homogeneous at a molecular level. As a result, there are some DNS-Cl rich micro-domains, in which the concentration of DNS-Cl is much higher than that in the bulk phase. Therefore, inner filtering or self-quenching effect of the system would be strong and the fluorescence emission would be weak. With the elongation of time, the aggregates of DNS-Cl dissociate gradually due to the addition of new solvent, and the solution becomes more homogeneous, and thereby the fluorescence emission increases. It is reported that hydrophobic-lipophilic interaction is one of the most important weak interactions between organic molecules and

is the main driving force for their aggregations in aqueous phase and water/organic mixture systems.[11,13-15] However, for present system, the reason for the aggregation might be just the opposite. The liophobic-hydrophilic property of the sulfonyl chloride group on the DNS-Cl molecule might be the main reason for the aggregation of DNS-Cl in acetone even though the whole DNS-Cl is hydrophobic-lipophilic in nature. It is the weak driving force that is responsible for the slow establishment of new equilibrium and for the fact that the equilibrium state is not stable and easily affected by changes in external factors.

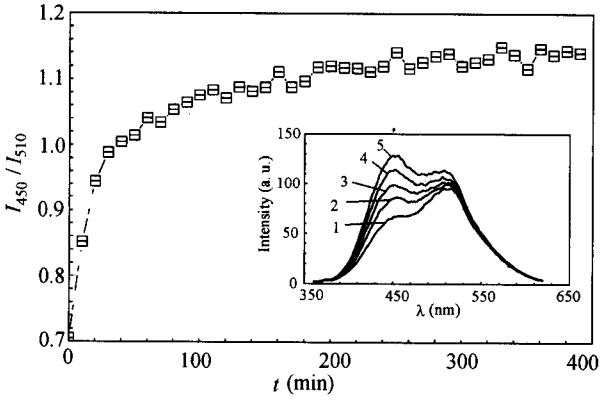

Fig. 4 Time dependence of the profile of the fluorescence emission spectrum of DNS-Cl in acetone after dilution with dry acetone (1: 0 min; 2: 10 min; 3: 30 min; 4: 110 min; 5: 390 min).

Effect of SDS *on the aggregation of* DNS-Cl *in acetone*

It has been reported that for the aggregation in aqueous phase and aqueous/organic mixture systems, introduction of some small molecules may enhance aggregation or may enhance deaggregation. The former kind of molecules is called enhancing aggregators and the latter one is called deaggregators. Normally, the enhancing aggregators are functioning by taking part in aggregation process.[11,15] As for the system of DNS-Cl in acetone, SDS shows obvious enhancing effect for the aggregation process. In Fig. 5, it may be noted that in the absence of SDS, the ratio of the monomer emission to the excimer emission (I_{450}/I_{513}) increases with time after diluting the saturated DNS-Cl solution with acetone. The increase reaches a constant value at about 16 h, indicating the establishment of a new equilibrium. For the system of high concentration of SDS (nearly saturated), the ratio decreases along with time. About 12 h later, the ratio reaches another constant value. The system with very low concentration of SDS behaves differently. The ratio increases slightly with time after introduction of the solvent. However, 10 h later, the ratio starts to decrease and a constant value is reached about 20 h later. These results show clearly that the introduction of SDS is favorable for DNS-Cl to exist in aggregated state. More work is needed to verify if SDS stabilizes the DNS-Cl aggregates by taking part in the aggregation.

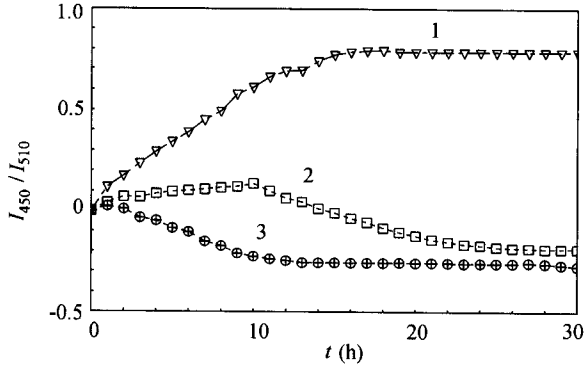

Fig. 5 SDS effect upon the profile of the emission spectrum of DNS-Cl in acetone (1: No SDS; 2: 1 : 8 saturated SDS solution in acetone; 3: 9 : 10 saturated SDS solution in acetone).

Conclusions

DNS-Cl is stable in acetone. No decomposition was observed during the process of purification and aggregation studies. DNS-Cl has a strong tendency to aggregate in acetone, and its [CAgC] is significantly lower than its solubility. The aggregation of DNS-Cl in acetone is reversible, and both the aggregation and de-aggregation are very slow . The introduction of SDS favors the formation and stabilization of the DNS-Cl aggregates.

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