Asian Journal of Chemistry Vol. 21, No. 7 (2009), 5299-5304

Fluorescence Quenching of Elsinochrome-A in Presence of Formamide

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Fluorescence quenching of elsinochrome-A in presence of formamide is reported here. It was found that the quenching observed was of static nature. It is also observed that quenching of the fluorescence of the indicator had a full reversibility. As it has a full reversibility and high sensitivity, a novel optical sensor for formamide can be constructed on this quenching.

Key Words: Fluorescence quenching, Elsinochrome-A, Formamide, Static nature.

INTRODUCTION

The development of sensors based on immobilized fluorescent reagents is a matter of growing interest[1,2]. Optical and fiber optical chemical sensors (FOCSs) have advantages over conventional sensors such as electrodes, *etc.* because they are simple, reliable, cost effective and relatively easy to maintain[3,4]. Fluorescence quenching refers to any process which decreases the fluorescence intensity of a certain fluorophore. A variety of processes can result in such a decrease in intensity such as collisional or dynamical quenching, static quenching, *etc*. Dynamic quenching (Fig. 1) results with collision between fluorophore in its excited state and quenching molecule. The fluorophore returns to ground state without emission of light. On other hand in static quenching, a non-fluorescent complex is formed between the fluorophore and the quencher. Usually only a fluorophore which is not complexed can exhibit fluorescence. As in both cases, the fluorescence intensity is related to the concentration of the quencher. Therefore, the fluorophore can serve as indicator for quenching agent. The dynamic fluorescence quenching can be described by the Stern-Volmer equation as follows:

$$I_0/I = \tau_0/\tau = 1 + K_d\{Q\} = 1 + K_q\tau_0\{Q\} \quad (1)$$

where, I_0 and I are the fluorescence intensities in the absence and presence of a quencher, respectively. K_d = quenching constant or Stern-Volmer constant, {Q} = concentration of the quencher and K_q = biomolecular quenching constant. τ_0 and τ are the lifetimes of the excited state of the fluorophore in the absence and presence of the quencher, respectively.

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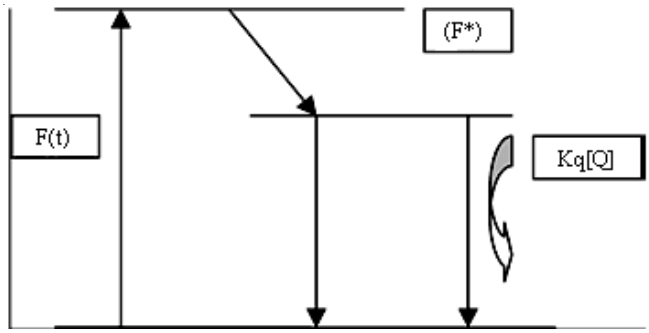

Fig. 1. Schematic diagram of collisional quenching

Since collision of the quencher with fluorophore occurs in its excited state, the life time of the excited state is reduced too. Dynamic fluorescence quenching is a diffusion process and therefore, is also influenced by the solvent viscosity and temperature. Static quenching can be represented as the equation similar to equation described earlier as:

$$I_0/I = 1 + K_s\{Q\} \quad (2)$$

The quenching constant K_s, is now identical with association constant of the complex formed between fluorophore and quencher. In case of static quenching a fraction of fluorophore is removed by complexation where as the fluorescence of the uncomplexed portion remains unperturbed. Therefore, lifetime of the excited state of fluorophore is unchanged. Hence, in case of complex formation there is a frequently change in absorption spectrum of the fluorophore, whereas collisional quenching only affects the excited state.

Elsinochrome-A (EA) belong to perylenequinone compound[5] and its structure[6-10] is shown in Fig. 2

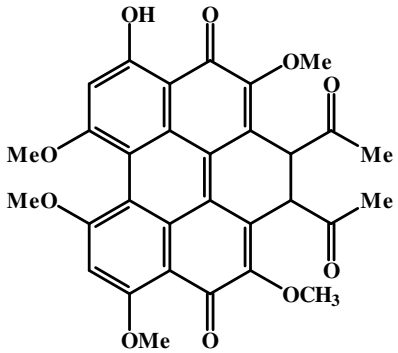

Fig. 2. Structure of Elsinochrome-A

In present study, Elsinochrome-A was selected as fluorophore because of its stability against the structural change with a change in environment *i.e.*, temperature,

solvent, concentration, *etc*. Formamide is a commercially used solvent of great importance as it has got a wide application in biomedical and chemical reactions. Exposure to moderate to high amounts of formamide can harm one's nerve system, respiratory system. High exposure may cause conjunctivitis and death. In US, the highest concentration allowed is 30 mg/m^3 (6.661×10^{-3} mol L^{-1}) in the air.

EXPERIMENTAL

All reagents are analytical grade and the millipore water is used all along. The temperature of experiment is 20 ºC. Elsinochrome-A (EA) was a gift from Prof. Weizhong Liu (Binzhou Medical university China). A solution of EA of 5.688 micromolar was prepared in different solvents.

A Perkin-Elmer LS55 fluorescence spectrometer (England) with a quartz cell of 1 cm path length is used to measure the fluorescence. UV spectra are recorded with a 756pc UV-Vis spectrophotometer (Shanghai spectrum instruments Co. Ltd., China). Elix10 + MilliQ pure water system (USA).

UV-Vis spectra of EA are measured in different solvent (Fig. 3). The fluorescence spectra of EA were measured in a series of solvent with the same concentrations (Fig. 4). Before measurements, tubes were shaken and placed into the thermostat water bath for 5 min. Then the assay solutions are transferred into the quartz cell and the fluorescence measurements are performed.

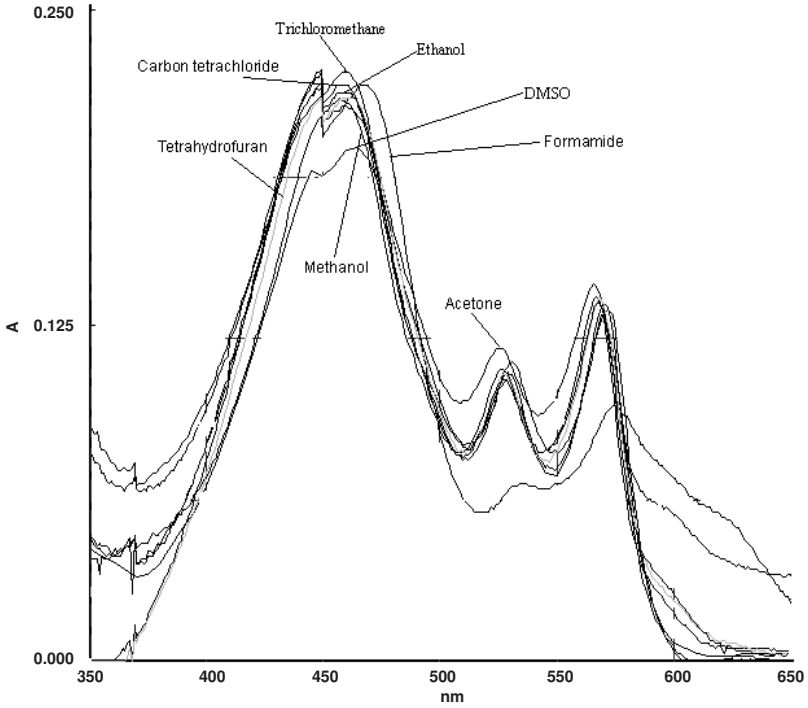

Fig. 3. UV-Vis spectrum of EA (5.688 μmol L^{-1}) in different solvent

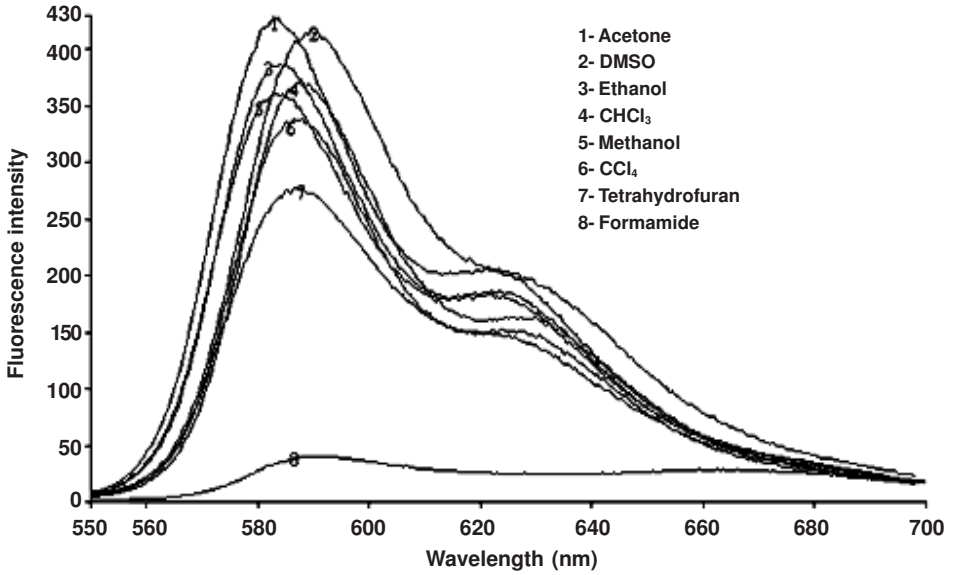

Fig. 4. Fluorescence spectra of EA (5.688 μmol L^{-1}) in different solvent

The fluorescence spectra of formamide-EA was measured in DMSO solvent (Figs. 5 and 6). The 480 nm excitation wavelength is used all along. The excitation slit and the emission slit are both 5 nm. The 500 nm/min scan speed is used all along.

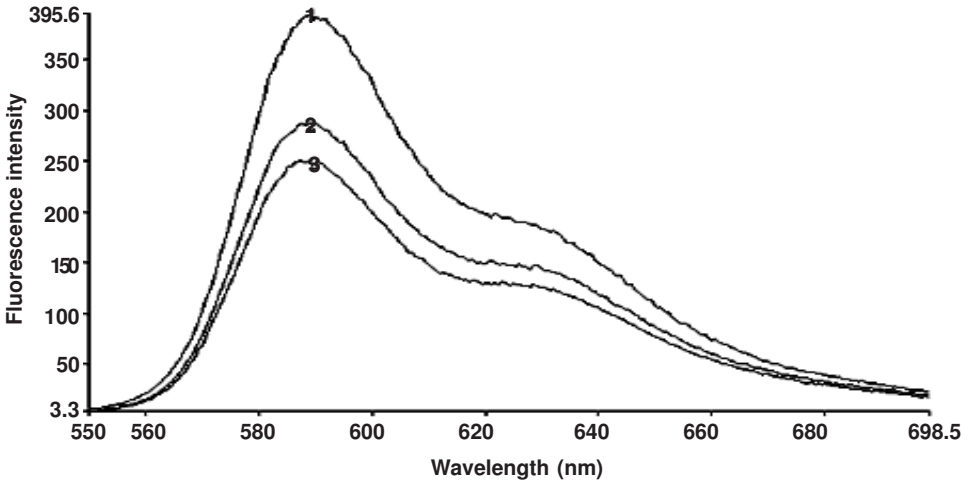

Fig. 5. Emission spectra of EA in presence of formamide in DMSO solvent, 1→3:0.5,10 μL formamide was added in 2 mL EA (5.688 μmol L^{-1}) solution

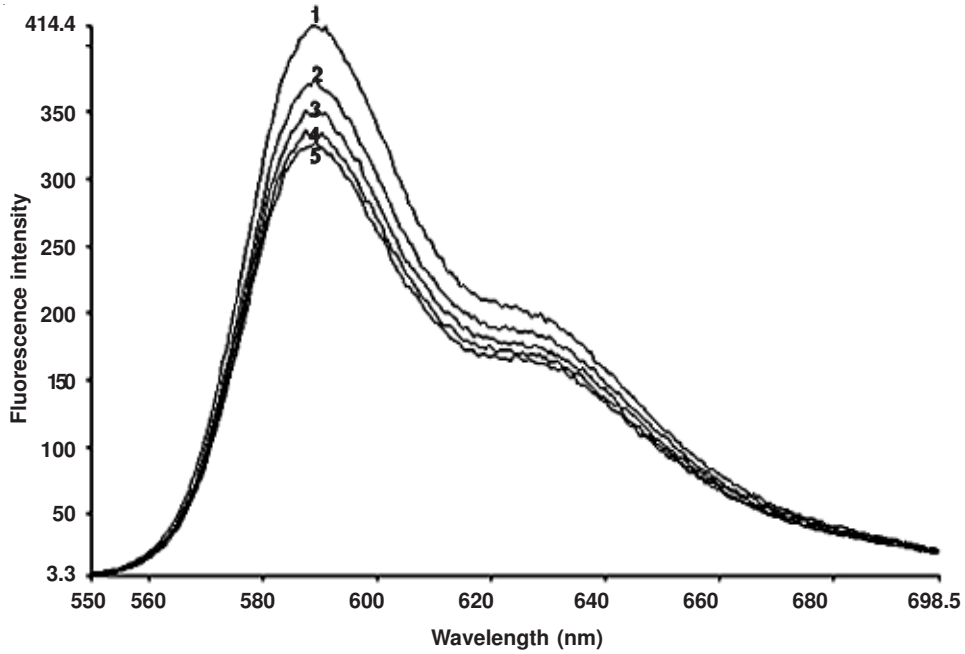

Fig. 6. Emission spectra of EA in presence of formamide in DMSO solvent, 1→5:0, 2.5, 5, 7.5, 10 µL formamide was added in 2 mL EA (5.688 µmol L^{-1}) solution

RESULTS AND DISCUSSION

UV-Vis spectra: The result of UV-Vis spectrum analysis of EA (5.688 µmol L^{-1}) in different solvent is shown in Fig. 3, red shift of EA are observed in the absorption spectra in formamide solvent and blueness shift in acetone solvent. The shift are not observed in other solvent.

Fluorescence spectra: The results of EA (5.688 µmol L^{-1}) fluorescence spectra in different solvent are shown in Fig. 4.

Fig. 4 shows that formamide is the best quencher to EA in the same concentration and the emission peak (588.68 nm) is the same as the DMSO. So the formamide is selected as the quencher to EA in DMSO solvent.

The quencher fluorescence spectra of formamide to EA in DMSO solvent is shown in Figs. 5 and 6. The result showed that the peak emission wavelength (588.68 nm) (Figs. 5 and 6) remains unaltered during the quenching experiments performed in DMSO solvent. It may be considered that the process of quenching is static in nature. This inference is supported by the fact that the change in absorption spectrum (Fig. 3) of EA is observed in presence of the formamide in DMSO solvent.

The most important is the sharp decline of fluorescence intensity caused by a little formamide (Figs. 5 and 6), for example, 5 µL formamide was added to 2 mL EA (5.688 µmol L^{-1}) solution (formamide concentration 5.536×10^{-5} mol L^{-1} < 6.661×10^{-3} mol L^{-1}). The decrease of fluorescence intensity is more than 100 (Fig. 5) and

it is also observed that quenching of the fluorescence of the indicator had a full reversibility. As it has a full reversibility and high sensitivity, a novel optical sensor for formamide can be constructed on this quenching.

The quencher plot (Fig. 7) shows a good linear relationship, $F_0/F = 1545.3\ [Q] + 1.076$.

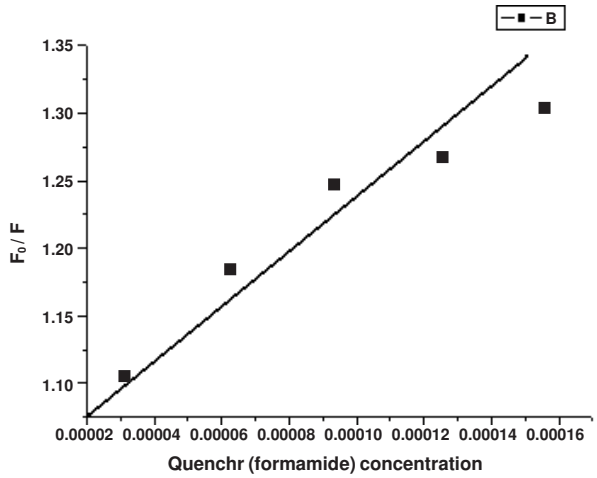

Fig. 7. Quencher plot of formamide to EA (5.688 μmol L^{-1}) in DMSO solvent

Conclusion

The quencher of formamide to EA may conclude that the quenching is of static nature, the value of $K_s = 1545.3\ L\ M^{-1}\ S^{-1}$. As it has a full reversibility and high sensitivity, a novel sensor can be constructed easily for its application for detection of formamide in vapour or solution form.

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(*Received*: 14 August 2008; *Accepted*: 30 April 2009) AJC-7469