Research Paper

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Investigations on The Interactions of Aurintricarboxylic Acid with 18-Crown-6: Analysis of Fluorescence Enhancement

Chemistry

KEYWORDS : absorption quenching, fluorescence enhancement, association constant

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ABSTRACT

The nature of interactions between aurintricarboxylic acid (ATA) and 18-crown-6 (CW) was investigated by measuring steady state absorption quenching spectra and fluorescence enhancement spectra in aqueous medium. 18-crown-6 (CW) is macrocyclic polyethers has an internal nanocavity, a diameter of ca. 2.6-3.2 Å. The fluorescence intensity of ATA increases significantly with the increasing concentration of CW producing 1:1 complex formation with association constant K~2.9 x 103 M-1. The absorption studies indicate that a ground state complex is formed between the probe ATA and the host CW in aqueous medium. In the other hand in the previous studies, it has been found that the fluorescence intensity is decreased markedly with increasing the concentration of CW in case of protonated tryptophan (Trp). These anomalous behaviours are discussed here by various spectroscopic techniques.

Introduction:

A number of studies have been carried out on the noncovalent inclusion complex with 18-crown-6 since 1980's, using fluorescence enhancement and quenching method which are closely related to the ground state or the excited singlet state of the chromophores in absence and presence (with the increasing of concentration) of 18-crown-6 [1-2]. The small molecular ions (which diameter are less than the internal ring cavity c.a. 2.6-3.2 Å) such as Na^+, K^+ etc are easily formed the complex in nonaqueous or aqueous media, but large organic molecular ions or molecules can be formed the complex with its in only in the aqueous media (or hydrophilic media) as loon pairs are oriented to the exterior in this medium [3, 4]. In the previous studies, it has been observed that the fluorescence intensity of the probe (which diameter are less than the internal ring cavity) is either increased or decreased markedly with increasing concentration of macromolecular host crown ether, which is known as fluorescence-enhancement or quenching [5]. Here the Aurintricarboxylic acid (ATA) is used as probe, and the properties of this complex formed with 18-crown-6 (CW) in aqueous media were studied by various spectroscopic method. The observations are compared with the anomalous behaviours of other organic chromophores.

Materials and Methods

Arintricarboxylic Acid (**ATA**) and 18-crown-6 (**CW**) purchased from Aldrich have been used directly from the package. The pH and resistivity of Millipore water are 6.8 and 18.2 Ω cm respectively. At the ambient temperature (296K) steady-state UV-vis absorption and fluorescence emission spectra of dilute solutions (10^{-5} M to 10^{-6} M) of the samples were recorded using 1 cm path length rectangular quartz cells by means of an absorption spectrophotometer (Shimadzu UV-VIS 2401PC) and F-4500 fluorescence spectrophotometer (Hitachi) respectively.

Results and Discussions

The steady state UV-vis absorption spectra of dye ATA exhibits the lower-energy absorption band systems in the region of 250 nm to 400 nm (broad peak ~ 310 nm) in aqueous solution (Millipore water at pH 6.8). Interestingly with the gradual addition of macrocyclic host CW host in that aqueous solution, the absorption of ATA gradually decreases (fig. 1), and

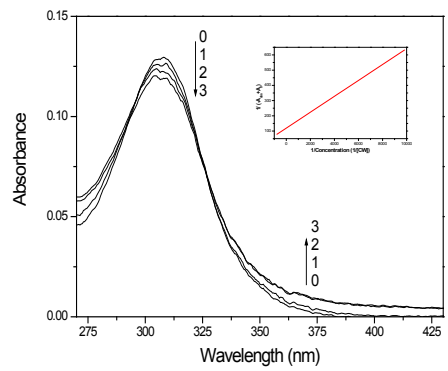

Figure 1: Steady state UV-vis absorption spectra of ATA in presence of increasing conc. of CW (Concentration of ATA ~ 3.1 x 10^{-5} M); [CW] ranges from (1) 0, (2) 3.83x 10^{-4}, (3) 2.83 x 10^{-3}, (4) 8.75 x 10^{-3}. **Inset**: Double reciprocal plot of absorption quenching for the systems: ATA + CW*. *indicates increasing concentration.

correspondingly, a weak broad absorption band at around 370-400 nm in higher wavelength region appears at high concentration of CW showing clearly a isobastic point. This formation of new absorption band across due to the formation of some ground state complex from the intermolecular interactions between the dye and macrocyclic host CW in aqueous medium.

Equilibrium constant of formation, k_A, of ground state complex can be calculated by the equation [6] below of 1:1 stoichiometry. $ATA + CW \leftrightarrow ATA \bullet CW$

$$k_A = \frac{[ATA \bullet CW]_{eq}}{[ATA]_{eq}[CW]_{eq}} \text{ and}$$

$$\frac{1}{(-A_{obs} + A_0)} = \frac{1}{(-A_c + A_0)} + \frac{1}{k_A(-A_c + A_0)[CW]}$$

Where the A_{obs} is observed absorption of ATA solution containing different concentrations of CW at 310 nm; A_0 and A_c are the absorbance of free ATA and the complex solution at the same concentration of free ATA. The fig (1) shows the plot for ATA with CW system, there is a good linear dependence of $1/ (A_{obs}-A_0)$ on the reciprocal concentration of macromolecule CW indicating 1:1 stoichiometry complex of ATA •CW. The linear plot shows $k_A \approx 2.89 \times 10^3$ M^{-1} of 1:1 stoichiometry.

The emission studies have been done on the dye ATA by excit-

ing it at 310 nm in presence of CW at its different concentrations at pH 6.8 (aqueous medium). Fig (2) shows that ATA has a strong fluorescence band at 423 nm when exciting at 310 nm. Gradual addition of CW (macrocyclic host) to ATA solution leads the enhancement in the emission intensity without any change in shape and peak positions. The environment around the fluorescence probe ATA becomes more hydrophobic with the increase in concentration of macromolecular host. One of the possible reasons for experiencing a hydrophobic environment (lesser polar) may be due to the encapsulation (or complex formation) cavity of hydrophobic nature.

In order to understand the binding properties of ATA-CW complex, the fluorescence enhancement of ATA with increase in concentration of CW are followed the modified form of the Benesi-Hildebrand relation [7]

$$\frac{I_f}{I_0} = \frac{1+(I_{ATA.CW}/I_0)k_A[CW]}{1+k_A[CW]}.$$

Where, I_f , I_0 & $I_{ATA.CW}$ are the emission intensities of ATA in the absence, at intermediate concentration of CW & of the complex. *[CW]* is the intermediate concentrations of CW used. The term k_A is the binding constant of the complex.

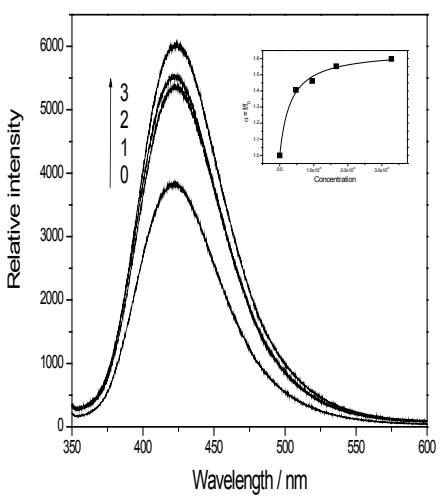

Figure 2: Fluorescence emission spectra of ATA (concentration ~ 1.53 x 10^{-5} M)
(λ_{ex}= 310 nm) in presence of increasing conc. of CW; [CW] ranges from 0, (2) 9.53 x 10^{-7}, (3) 1.66 x 10^{-6}, (4) 3.28 x 10^{-6} M. Inset: plots of fluorescence enhancement factors ($\alpha = f/f_0$) vs. concentrations of enhancer and its fitting curve. System: ATA + CW*.

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In Fig. 2, $\frac{I_f}{I_0^f}$, the enhancement factor, α vs. [Q] are plotted. Non-linear least square regression method is used for data analysis and the fluorescence data is made to fit into equation above. A satisfactory fit are obtained with $r^2 > 0.99$. The association constant of complex formation, k_A, is obtained ~ 3.1x 10^3 M^{-1}. by this method, indicates about 1:1 complex formation. Interestingly, the obtained value of k_A from these two method are nearly similar, i.e. the absorption quenching method and fluorescence enhancement method are equivalent to study the stoichiometry of the complex.

The free energy change (T=298K), can be calculated by the relation:

$\Delta G = -RT \ln k_A \approx$ -19.87 kJ/mol. This indicates strong binding between the two partners and the process is spontaneous [8, 9].

In the previous studies [4], the choice of solvent in this studies of crown ether complex are very much important. The cavity size of 18-crown-6 are near about 2.6-3.2 Å in aqueous media. So the molecules of large diameter than CW cannot directly entre into the cavity. For these molecules to form complex with 18-crown-6, the better choice of solvent is water, a hydrophilic medium. The conformation of crown ethers in non polar organic solvents reflects a "droplet of water in oil" with the lone pairs pointing to its interior. In water, or general speaking hydrophilic media, the loan pairs are oriented to the exterior, in advantageous for coordination with probe molecules of larger diameter than crown ether like tryptophan, aurintricarboxylic acid etc. The similar enhancement in fluorescence spectra were observed for Tryptophan -18-crown-6 complex (Trp•CW) but opposite behaviour were observed for complex of $TrpH^+$•CW complex [5]. $TrpH^+$•CW complex form a doumble shaped three dimensional structure, by which the excited indole moiety of $TrpH^+$ relaxed the excitation energy by vibrational deactivation through crown ether. In aqueous media, the excited singlets of ATA molecules relax majorly through vibrational deactivation of own. But in case of this new system, due to the complex formation with crown ether at least two or more carboxylic group closely formed hydrogen bond with the exterior pointing lone pair electron of oxygen atom of crown ether. Again the other counterpart of carboxylic group of ATA formed the other O....H bond with water molecules. So a closed pack structure is formed by this complex formation, the quenching of absorption and enhancements of fluorescence spectra have been occurred.

Conclusion:
The unusual phenomenon of fluorescence enhancement of the probe ATA are observed here due to binding with CW in aqueous medium. Both the steady state absorption quenching method and the fluorescence enhancement method support about the strong binding within the probe ATA and the macromolecular host CW in aqueous medium about the binding constant ~ 2.89-3.1 x 10^3 M^{-1} of 1:1 stoichiometry. These observation also inform us that the capability of exterior binding of the large probe molecule (diameter of probe > diameter of host molecule CW) with the crown ether is possible only in the aqueous medium. Using this fluorescence enhancement one can measure the quantities presence of the probe molecules in that aqueous medium.

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