Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 151 (2015) 662–666

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

The effect of dimethylsulfoxide on absorption and fluorescence spectra of aqueous solutions of acridine orange base

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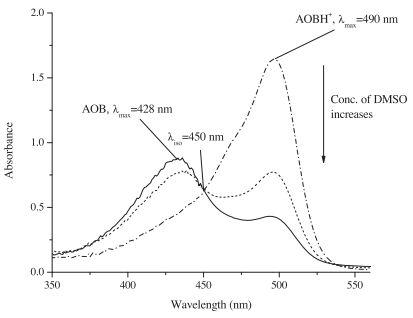
HIGHLIGHTS

- The effect of dimethylsulfoxide on protonation of acridine orange base was investigated.
- DMSO prevents the protonation of acridine orange base.
- The concentrations of neutral and protonated species were determined.
- The fluorescence intensity increases with the addition of DMSO.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 May 2015
Received in revised form 22 June 2015
Accepted 23 June 2015
Available online 7 July 2015

Keywords:
Acridine orange
DMSO
Acid–base equilibrium
Isosbestic point
UV–vis
Fluorescence spectroscopy

ABSTRACT

The photophysical properties of aqueous solutions of acridine orange base (AOB) in wide concentration range of dimethylsulfoxide (DMSO) were studied by using absorption and steady-state fluorescence spectroscopy techniques at room temperature. The absorption spectrum of acridine orange in water shows two bands at 468 and 490 nm which were attributed to the dimer ($(AOBH)_2^{2+}$) and monomer ($AOBH^+$) species respectively. In DMSO solution for the same AOB concentration only the basic form was detected with the band at 428 nm. The addition of DMSO to AOB aqueous solution leads to the decrease of absorption band at 490 nm and the new absorption band increases at 428 nm due to deprotonated (basic) form of AO and the first isosbestic point occurs at 450 nm. The evolution of isosbestic point reveals that an other equilibrium, due to the self-association of DMSO molecules takes place. From the steady-state fluorescence spectra Stokes shifts were calculated for AOB in aqueous and DMSO solutions. The addition of DMSO into the aqueous solution induced the enhancement in the fluorescence intensity of the dye compared to those in water.

1. Introduction

The study of AOB in aqueous solutions of DMSO is important from both practical and fundamental viewpoints. AO as a dye molecule is widely used in various technological applications [1,2]. In addition AO has a biomedical interest due to its ability to interact with genetic materials. In this respect it should be noted that the interaction of AO with nucleic acids is important problem to reveal biological actions of AO such as photodynamic and mutagenic actions [3,4]. On the other hand, the biomedical significance of DMSO is well known [5,6]. Therefore, it is expectable that the use of AO together with DMSO will open up the new avenues in the investigation of the mechanism of biomedical action of these compounds.

Acridine orange (AO) is a cationic dye, it is protonated at a pH lower than 10, even in just aqueous solutions. An important property of AO is that its spectrum does not agree with Lambert–Beer's

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http://dx.doi.org/10.1016/j.saa.2015.06.126
1386-1425/

law due to molecular aggregation as a result of strong dipole–dipole interaction in aggregate units. It should be noted that the aggregates, particularly dimers compare to the monomer show different photophysical and spectroscopic properties. In general, photophysical and spectroscopic properties of dye molecule depend upon the aggregation type. There are numerous spectroscopic studies regarding self-association and protonation of AO in aqueous solutions [7–9]. It is known that at low concentrations ($1–5 \times 10^{-5}$ M) in water there is an isosbestic point at about 472 nm corresponding to a protonated monomer–dimer equilibrium over this concentration range [9,10]. It should be noted that longer aggregates are formed at high concentration of AO (>10^{-4}).

The association was studied in aqueous solutions of some non-electrolytes such as methanol, ethanol, dioxan, urea as well [8]. Particularly, in ethanolic solution acid–base equilibria for both water and ethanol have been considered [11]. In [11] the spectral behavior of neutral AO in water and ethanol has been investigated. It is interesting to mention that the addition of anhydrous potassium carbonate to AO in ethanol leads to the formation of free base AO. Thus according to [11] with the addition of potassium carbonate free AO is generated by the removal of water. In this respect it should be mentioned that DMSO has unique property to form strong H-bonding with water molecule. Moreover, self-association of DMSO molecules occurs even in aqueous solutions [12]. Therefore it is expected that DMSO will prevent protonation of AO in water and it can serve as a model to govern equilibrium between different species including free base, protonated monomer and dimer of AO.

2. Experimental

2.1. Materials

Acridine orange base (AOB) was obtained from Sigma–Aldrich, USA and was used as received. DMSO was purchased from Alfa Aesar (99.9%), Germany. AOB was dissolved in DMSO and double distilled water (conductance less than 2 μs cm^{-1} at 25 °C) and the desired concentration of solutions was obtained by dilution (about 4.31×10^{-5} M for UV–vis measurements and 1.1×10^{-5} M for fluorescence measurements).

2.2. Methods

The absorption spectra were recorded using Specord 50 PC equipment, for fluorescence measurements Varian Cary Eclipse fluorescence spectrophotometer was used with excitation wavelength 425 nm for all the solutions. The fluorescence emission spectra were recorded in the range of 430–800 nm. The path length used in absorption and emission experiments was 1 cm. All experiments were carried out at 20 °C.

3. Results and discussion

Fig. 1 shows the molecular structures of AOB and its protonated form $AOBH^+$. To study the behavior of AOB in DMSO-water solution first absorption and fluorescence spectra of AOB were recorded in water and DMSO. The normalized absorption and fluorescence spectra in water are shown in Fig. 2(a). The absorption spectrum shows two maxima at 468 and 490 nm which can be assigned to the dimeric ($(AOBH)_2^{2+}$) and protonated monomeric ($AOBH^+$) species respectively [9,10]. For the protonation of AOB and the dimerization of $AOBH^+$ the following equilibria are proposed:

$$AOB + HOH \Longleftrightarrow AOBH^+ + OH^- \quad (1)$$

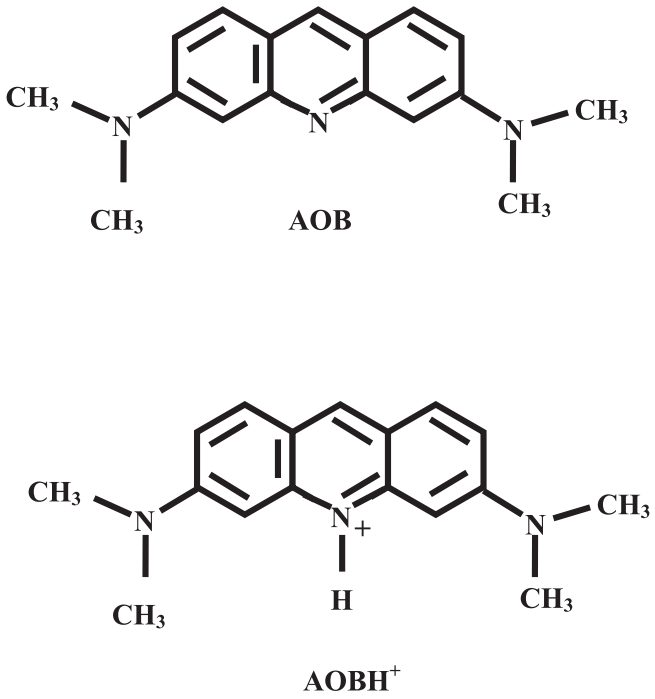

Fig. 1. Molecular structures of acridine orange base and protonated monomer.

$$2AOBH^+ \Longleftrightarrow (AOBH)_2^{2+} \quad (2)$$

It is important to note that the used concentrations of AOB were about 10^{-5} M and hence longer aggregates do not form [8]. The emission spectrum of AOB aqueous solution shows maximum at 529 nm, which is in good agreement with results reported previously [10] as for the detection of fluorescence of dimeric form the initial concentration of AOB must be higher than 1×10^{-3} M. The normalized absorption and fluorescence spectra of AOB in DMSO were also recorded and presented in Fig. 2(b). Absorption spectrum shows one maximum at 428 nm and emission band at 525 nm, which corresponds to the basic form of acridine orange and are similar to that reported for the basic dye [13].

3.1. The effect of DMSO on absorption spectra

The addition of DMSO to aqueous solution of AOB caused a remarkable change of AOB absorption spectrum as illustrated in Fig. 3. At the beginning when a small amount of DMSO has been added the absorption band at 490 nm due to $AOBH^+$ form increased whereas the absorption band of $(AOBH)_2^{2+}$ at 468 nm decreased and appeared as a shoulder (curves b and c in Fig. 3).

The further increase of DMSO concentration leads to the decrease of absorption band at 490 nm and the new absorption band increases at 428 nm due to deprotonated (basic) form of AO, as was mentioned above. It is known that absorbance of different dilute solute species is additive. Any shift in the equilibrium between two interconverting species redistributes the weights of their contributions changing the net spectrum [14]. In our case the first isosbestic point occurs at 450 nm within range of molar fraction of DMSO from 0.17 to 0.68. With the addition of DMSO content the second isosbestic point at 443 nm is generated. The presence of first isosbestic point clearly indicates that there is an equilibrium in (1) reaction and the addition of DMSO leads to the formation of basic form. The second isosbestic point indicates that an other equilibrium, due to the self-association of DMSO molecules [15] takes place. It is known that for the higher concentration