

Acid-base equilibria studies and photophysics in AOT w/o microemulsions of (bi)functionalized benzo[*a*]phenoxazinium chlorides

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Abstract: It is reported the fluorescence and absorption spectra of pure cationic and neutral forms of benzo[*a*]phenoxazine derivatives containing different functional groups at 5- and 9-positions obtained by shifting the acid-base equilibria with the addition of a strong acid or base. The photophysical behavior of these compounds in AOT/cyclohexane/water w/o microemulsions as a function of water content is also investigated.

Keywords: Benzo[*a*]phenoxazines, acid-base equilibria, AOT and microemulsions

1. Introduction

In the recent years, fluorescent dyes are used as probes for the detection of heavy metal ions, amino acids and other biomolecules.¹⁻⁴ Among the fluorescent dyes, Nile blue and its derivatives, which belong to the class of benzophenoxazines, have gained considerable attention due to their profound applications as molecular probes in various biological systems. In fact, they fluoresce at near the far visible-near infrared region with minimum interference from absorption scattering and reduced risk of photodecomposition. Owing to their importance, various new analogues of the benzophenoxazines with improved water solubility and functional groups that allow for covalent linkage to the analyte were developed.^{5,6-9} Considering these facts, and in continuation of our research interests in the synthesis and characterization of the organic fluorophores,⁶⁻¹³ we herein report the photophysical studies including the acid-base equilibria and the behavior in w/o microemulsions of five benzo[*a*]phenoxazinium chlorides with different combinations of substituents at 5- and 9-positions of the heteroaromatic system.

2. Results and discussion

Benzo[*a*]phenoxazinium chlorides **1a-e** were synthesized by condensation of 2-nitrosophenol hydrochlorides with *N*-naphthalen-1-amine derivatives in the acid medium (Figure 1).¹⁴ The required nitrosophenol was obtained by nitrosation of the corresponding 3-aminophenol precursor with sodium nitrite and hydrochloric acid in water with ethanol as the solvent.⁶⁻¹⁵

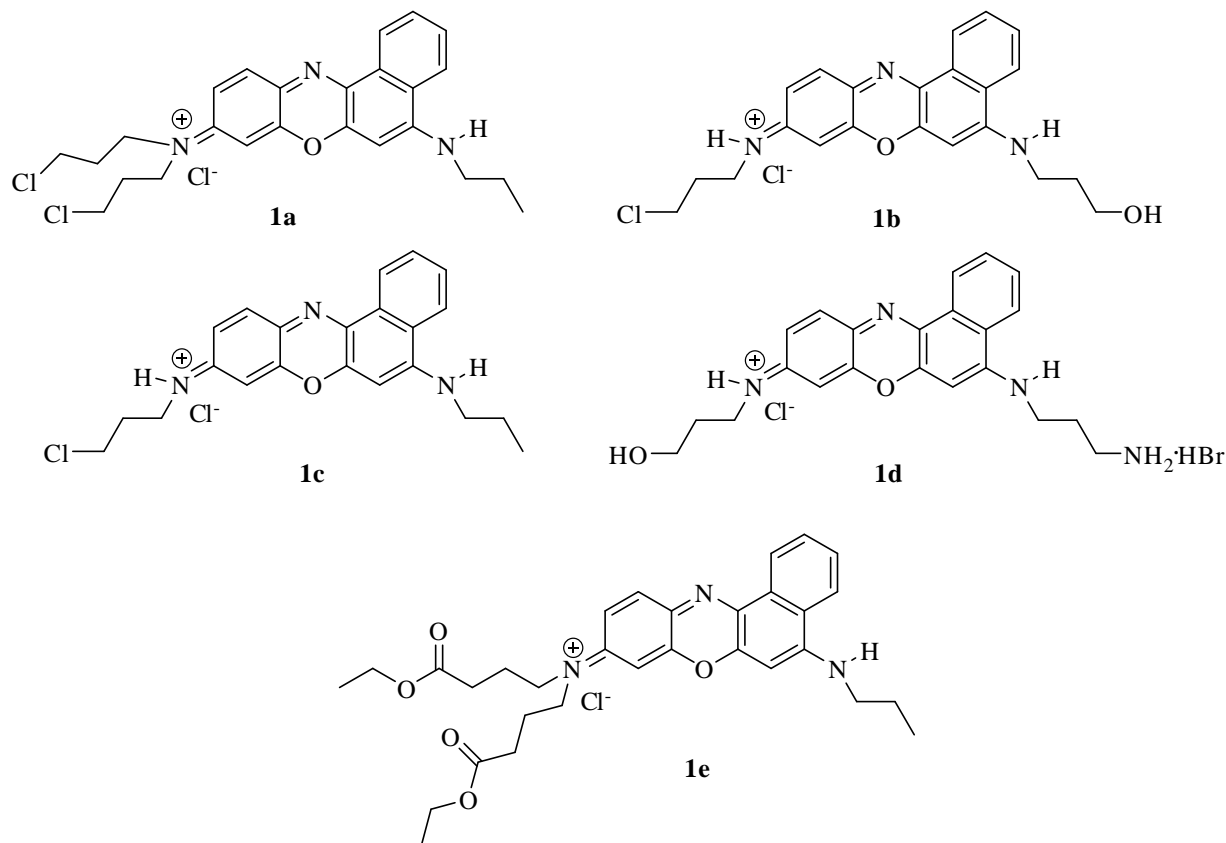


Figure 1. Structures of (bi)functionalised benzo[*a*]phenoxazinium chlorides **1a-e**.

Previous studies of this type of compounds showed that its photophysics in proton-accepting solvents is influenced by acid-base equilibria mainly located at the 5-amino position.^{11,13} In ethanol media the absorption spectra is dominated by an acidic form (AH⁺) and a ~100nm blue shifted neutral form (A).⁶ The fluorescence of the basic form is broad and centred at around 600nm whilst the acid form (AH⁺) shows a band centred above 660nm with a much higher quantum yield (~0.4).⁶ These fluorescence bands are seen to red shift when the medium changes from ethanol to water (data not shown). This is typical of π - π^* electronic transitions.

At 470nm the basic form is mostly excited with a small fraction of acidic form. At 575nm the situation is reversed. In Figures 2 to 6 absorption and fluorescence of compounds **1a-e** are shown. The above characteristics

are confirmed in ethanol media (dashed lines) when acidified with Trifluoroacetic acid or basified with Tetraethylammonium hydroxide (TEAH). A special feature is observed for compounds **1b**, **1c** and **1d** (Figures 3 to 5) for which an additional emission band centred at ~540nm appears for the acid form fluorescence when excited at 470nm. The structural feature that distinguishes all these compounds from the others (**1a** and **1e**) is the monoalkylated at the 9-amino position.

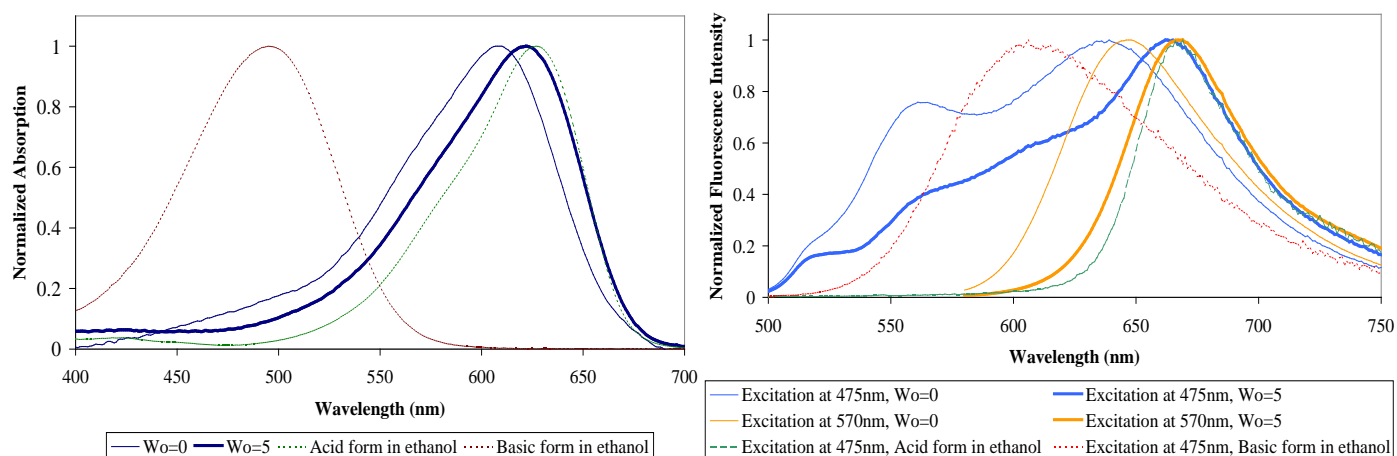


Figure 2. Absorption and fluorescence spectra of compound **1a** in AOT/cyclohexane reverse micelles ($W_o=0$ and $W_o=5$) and in acidified and basified ethanol media.

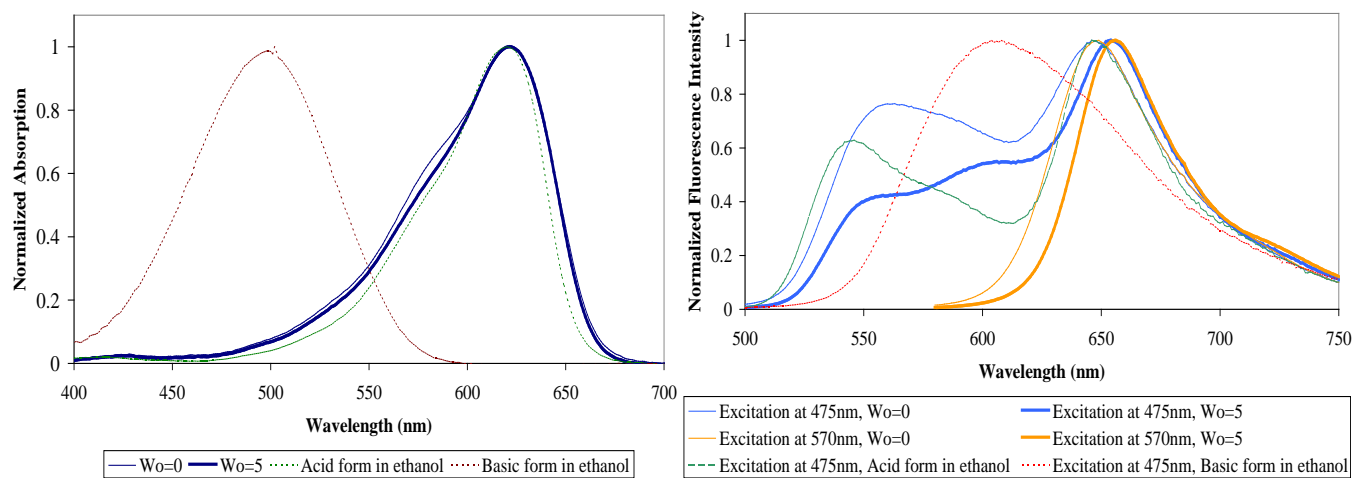


Figure 3. Absorption and fluorescence spectra of compound **1b** in AOT/cyclohexane reverse micelles ($W_o=0$ and $W_o=5$) and in acidified and basified ethanol media.

In a reverse micellar system nanosized water pools are surrounded by a layer of surfactant (AOT) molecules. This organization allows the water phase to be homogeneously dispersed in the organic (cyclohexane) solvent. These nano-pools have been used as chemical reactors allowing, by confinement effects, the synthesis of nanoparticles.¹⁶ Depending on the pool size of the reverse micelles, the water within them has different properties than “bulk” normal water. The size of the reverse micelles is determined by the W_o parameter that is defined by the ratio of water to surfactant concentrations.¹⁷

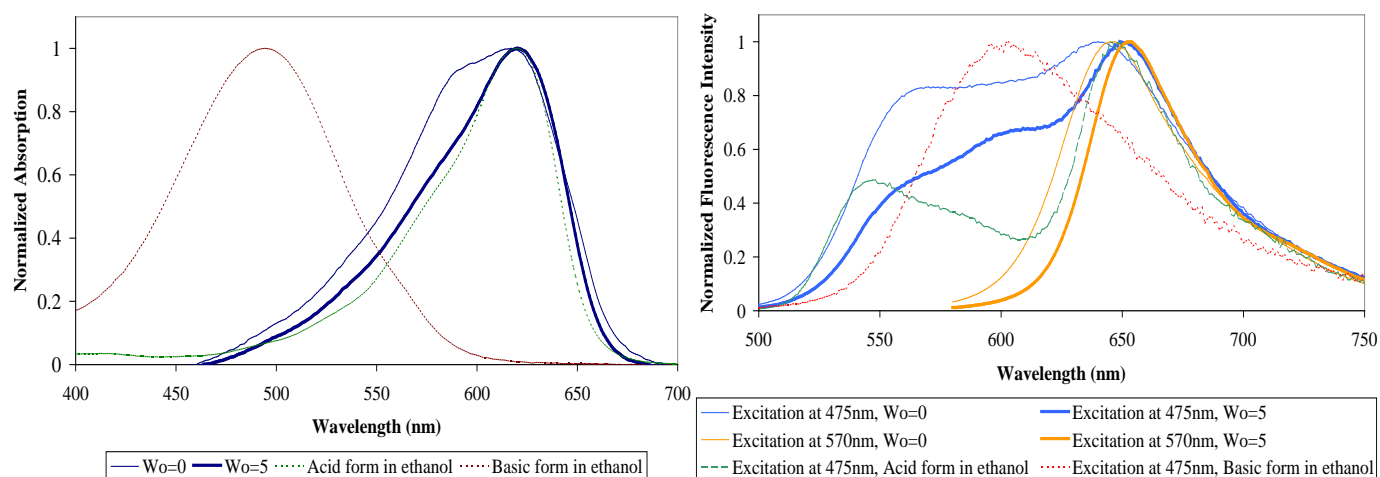


Figure 4. Absorption and fluorescence spectra of compound **1c** in AOT/cyclohexane reverse micelles ($W_o=0$ and $W_o=5$) and in acidified and basified ethanol media.

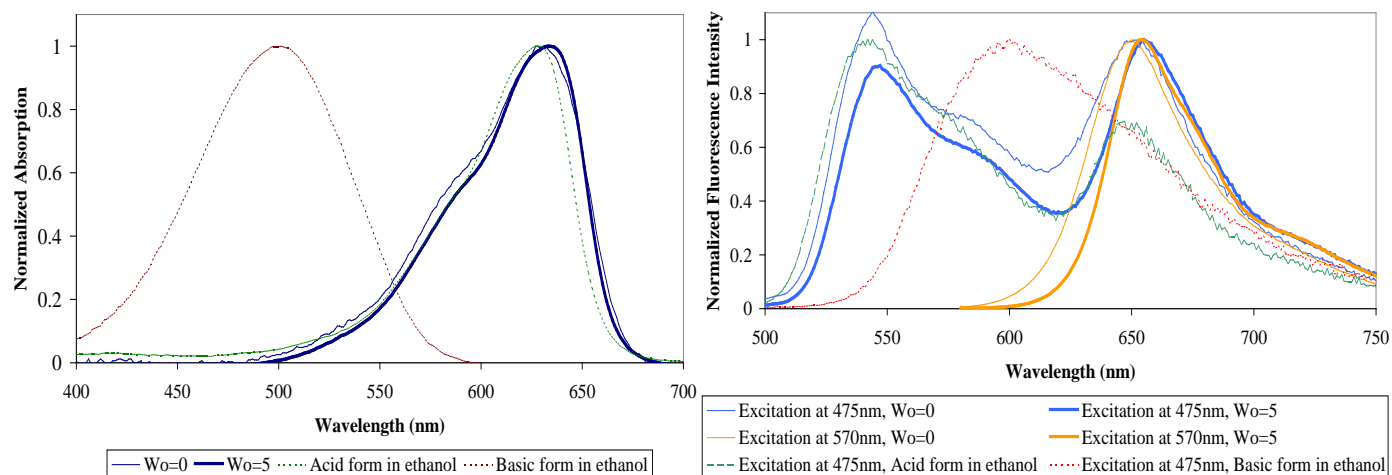


Figure 5. Absorption and fluorescence spectra of compound **1d** in AOT/cyclohexane reverse micelles ($W_o=0$ and $W_o=5$) and in acidified and basified ethanol media.

It is expected that the synthesized benzo[*a*]phenoxazinium chlorides **1a-e** have the potential to probe the water environment in the reverse micelles because of the already discussed dependence of its photophysics on an acid-base equilibria and of a slight solvatocromism through a π - π^* electronic transition. As an initial study, Figures 2 to 6 show the absorption and emission spectra of the compounds **1a-e** in AOT/cyclohexane reverse micelles at $W_o=0$ (absence of water) and $W_o=5$.

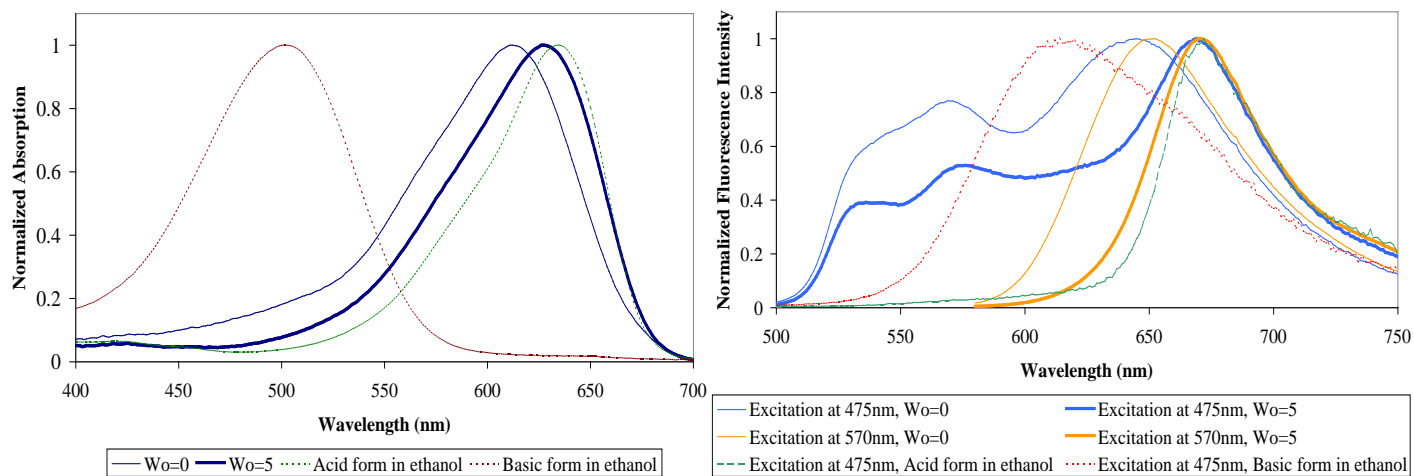


Figure 6. Absorption and fluorescence spectra of compound **1e** in AOT/cyclohexane reverse micelles ($W_o=0$ and $W_o=5$) and in acidified and basified ethanol media.

For compounds **1a** and **1e** a red shift is observed, both for absorption and fluorescence of the acid form, when the water content increases. For $W_o=5$ the red side of the absorption spectra and the fluorescence of the acid form coincide with what is observed in ethanol media. This indicates that the compounds are at the interface and not in the water pool. For compounds **1b**, **1c** and **1d** only a disappearance of a blue shoulder in the absorption spectrum and a smaller red shift of the acid fluorescence spectra occur. This shoulder has been previously reported as originating from H-aggregates.⁶ When excited at 470nm fluorescence can occur both from the acid and basic forms. The fluorescence spectrum below 640nm is dominated by the basic form (maximum at ~600nm) but an additional band near 550nm appears for all compounds. For compounds **1a** and **1e** a further band occurs near 520nm. These additional bands always decrease with the increase of W_o . The band near 550nm appears for all compounds and not only for compound **1b**, **1c** and **1e** as seen in acidified ethanol. This means that mono-alkylation at the 9-amino position is not the key factor for this additional emission as was discussed above. The fact that no corresponding absorption changes are

observed is an indication that this blue shifted fluorescence bands originate from excited state processes.

3. Conclusions

The photophysics of the acid and basic forms of five benzo[*a*]phenoxazinium chlorides bearing different functional groups and 5- and 9-amino positions were studied in ethanolic media by adding either a strong acid or a strong base. The behavior of these compounds in AOT/cyclohexane w/o microemulsion allows the study of water properties in the reverse micelles as they locate in its interface and their absorption and fluorescence spectra depends on the water content through the W_o parameter.

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