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Spectroscopic characterization of thionine species in different media

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Abstract

The influence of pH on aggregate formation and changes in the structure of thionine monomers in solution has been determined. Spectroscopic data (UV−vis and ^{1}H NMR) revealed that a free base form of the dye is formed at basic pH values, which displays highly hydrophobic behavior. The cationic monomeric form can be photoreduced in both organic and aqueous solution, while the free base form did not show any photoactivity in such media.

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1. Introduction

The iron−thionine system is a redox system with promising use in photogalvanic cells [1,2]. The reaction between thionine (Thio) and Fe(II) is used to convert light into chemical energy. Because the Thio monomer is the only photoactive species, its aggregation and polymerization have a significant effect on the photophysical properties of this cell. Aggregation is directly related to both the concentration of Thio and the chemical nature of the solvent [3]; at low concentration there is a peak at 597 nm due to monomeric species. At high concentrations, a second peak on the lower wavelength side of the 597 nm peak appears that corresponds to aggregate formation [4]. Many investigations have studied the physicochemical properties of Thio, mainly focusing on its capabilities as a dye for solar cells. Different organized media, such as micelles and microemulsions, have been also used to improve the output voltage of the iron−Thio cell [5]. It was found that a surfactant media inhibits the reverse homogeneous redox reaction and so avoids Thio aggregation and polymerization. Other research groups also described the photophysical properties of this dye which has been modified with macromolecules [6], giving a hydrophobic environment to the dye. This change resulted in excited states with longer lifetime, and the corresponding changes in the efficiency of the cell. Mathai et al. [7] investigated the photogalvanic effect in the iron−thionine system, under different conditions, including concentration of reactants and pH. Although proton concentration is known to play an important role in the efficiency of the photoinduced reaction, detailed analysis of the effects of pH and solvent on solution composition has been rarely reported [8]. Moreover, previous works do not consider the acid−base properties of thionine at high pH values. In this paper, the influence of pH on the aggregation and structure of thionine in a wide pH range is reported. Also the photoinduced reduction of thionine under those conditions is analyzed. In order to characterize the effects of the observed acid−base behavior, solubility and redox reactions in different solvents were studied using UV−vis and ^{1}H NMR spectra.

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2. Experimental

Aqueous solutions were prepared using ultrapure water (Milli Q − Millipore). Diaminophenothiazine chloride (thionine) solutions were prepared with different electrolytes (HCl or NaOH) depending on the pH required, as indicated in the text for each particular case. Organic solutions were prepared using 1,2-dichloroethane (1,2DCE; Dorwil) as solvent. Tetraphenylborate was employed as reducing agent for Thio in 1,2DCE; for this, tetrapentylammonium tetraphenylborate (TPnATPB), which is highly soluble in 1,2DCE, was the hydrophobic salt used. Due to the overlapping aromatic resonances between tretraphenylborate and Thio, sodium sulfite (Mallinckrodt; Chemical Works, New York) was used as reducing agent in deuterated methanol. In order to favor the reduction reaction, an excess of sodium sulfite was added to the methanolic solution of Thio.

The UV−vis spectra of both organic and aqueous solutions were recorded using a Shimadzu UV-1601 spectrophotometer. ^{1}H NMR spectra were obtained with a Bruker AC-200 spectrometer at 200.13 MHz in deuterated acidic and alkaline methanolic solutions of Thio. Alkaline conditions were obtained by the addition of NaOD 40 wt%. Chemical shifts are given in ppm (δ) downfield from TMS internal standard.

3. Results

In order to analyze the effect of pH on aqueous solutions of Thio, UV−vis spectra were recorded. Fig. 1a shows the UV−vis spectra of Thio at several pH values. As can be observed, a clear absorbance peak was obtained at 599 nm. This peak has been attributed to the presence of the Thio monomer with a positive resonant charge [4]. As pH increases, the magnitude of this peak decreases and a new peak appears at 510 nm indicating a pH-dependent interconversion of species. A plot of the absorbance at 599 nm vs. pH (Fig. 1b) shows a change in slope suggesting a pK_a value of around 8 for this acid−base reaction. This result was confirmed by titration of an aqueous solution of Thio with NaOH and is in good agreement with previously reported values, obtained from electrochemical experiments [9]. It was also observed that the solubility of the dye in water decreased significantly with increasing pH; indeed, the dye is scarcely soluble at pH > 12. Aggregation of the cationic Thio and/or precipitation of the basic form of the monomer could be responsible for this dependence of solubility with the pH.

Considering that aggregate formation is inhibited in organic solvents and the low solubility in water at high pH values, the behavior of Thio in 1,2DCE was analyzed. As ThioCl is not soluble in organic solvents, the 1,2DCE was saturated by agitation with either acidic (pH = 2) or alkaline (pH = 10) aqueous Thio solutions, obtaining solutions **A** and **B**, respectively. Higher amounts of solute were dissolved and the color was much more intense in **B** than in **A**. These results suggest that the presence of OH$^-$ shifts the Thio acid−base equilibrium towards the formation of a conjugated base, highly soluble in the organic solvent. The UV−vis spectra of solutions **A** and **B** are shown in Fig. 2. Solution **A** shows an absorbance

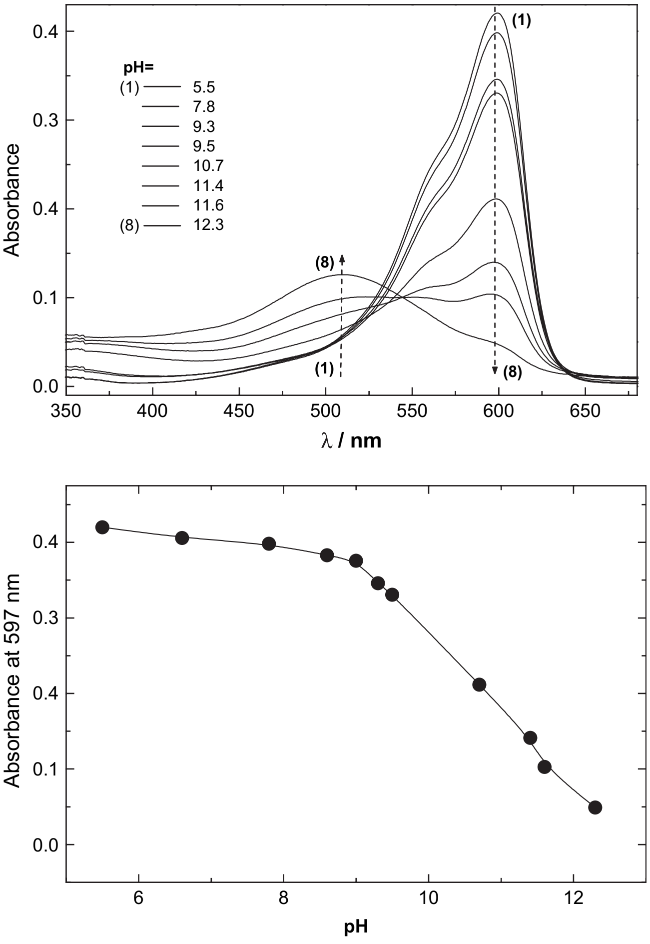

Fig. 1. (a) UV−vis spectra of Thio in aqueous solutions at different pH values. $C_{\text{Thio}} = 5.0 \times 10^{-4}$ M. The pH of the media was adjusted with a few drops of NaOH; 1 M. Spectra were corrected with dilution factors. (b) Absorbance of Thio at 597 nm as a function of pH.

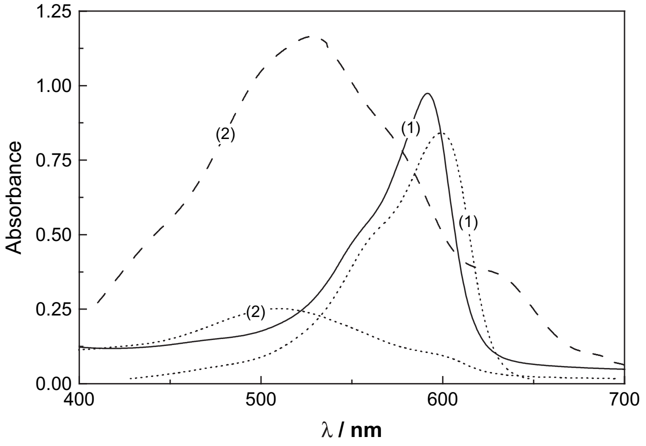

Fig. 2. UV−vis spectra of thionine in organic phase (1,2DCE), after extraction from an aqueous solution at acid (solid line) and basic (dashed line) pH values. As a matter of comparison, the scaled absorbance spectra of the aqueous phase before extraction are shown in dotted line.

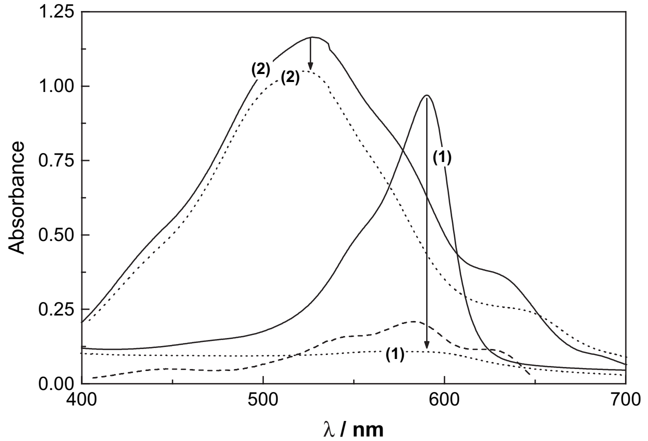

Fig. 3. Effect of illumination on the UV–vis spectra of thionine in organic phase (1,2DCE), after extraction from an aqueous solution at acidic (1) and basic (2) pH values. Dark spectra (solid line) and illuminated spectra (dotted line). The difference spectrum obtained from those after extraction at basic pH value is depicted as dashed line.

peak at 599 nm and a shoulder at 550 nm while solution **B** shows a wide absorbance peak with its maximum located at 520 nm. For comparison purposes, the spectra of Thio in acid and basic aqueous solutions are also included in Fig. 2 (dotted line). As can be observed, the saturation of 1,2DCE with acidic or basic solutions produces the dissolution of the same species than in aqueous solutions.

It is very well known that the monomeric form of Thio is able to participate in photoinduced chemical reactions with different electronic donor compounds. In order to analyze this photoactivity, both the A and B forms of Thio in organic solutions were analyzed. Photoactivity was measured in the presence of tetraphenylborate anion, a well known reducing agent in organic solvents [10], by recording the UV–vis spectra after illumination of the **A** and **B** solutions (Fig. 3). The absorbance of solution **A** decreased and almost vanished after illumination indicating that the solution comprises mostly the cationic form of Thio (Thio$^+$). In contrast, the absorbance of solution **B** decreased only slightly; in this case, it is important to point out that the difference between the two spectra (without and with illumination) had the same shape as that of solution **A**. This finding is clear evidence for the presence of just a small amount of Thio$^+$ in the organic solvent saturated with an alkaline solution and that the main compound is not photoactive.

Table 1
^{1}H NMR spectral data of **I**–**III**[a] (see Fig. 4 for structures)

H	**I**[b]	**II**[c]	**III**[d]
x–x′	8.05 d (9.5)	7.33 d (9.5)	7.29 d (8.8)
y–y′	7.34 dd (9.5, 2.2)	6.87 dd (9.5, 2.2)	6.72 d (8.8)
z–z′	7.22 d (2.2)	6.70 d (2.2)	6.77 br s

[a] Chemical shifts (δ) downfield from TMS; 200.13 MHz; *J* coupling (in parentheses) in Hz.
[b] CD_3OD.
[c] CD_3OD–ODNa 40% (98:2).
[d] D_2O–CD_3OD (60:40).

These results indicate that the insoluble compound present in aqueous, alkaline solutions is the conjugate base of Thio$^+$ and that there is no aggregate formation; the higher solubility in the organic phase with the same absorption spectra than in the aqueous phase supports this proposal.

In order to analyze the chemical nature of the species involved in the acid–base reaction, ^{1}H NMR spectra were obtained for acidic and alkaline solutions of Thio in different solvents. As the peaks in water were too wide due to aggregate formation, ^{1}H NMR spectra were performed in MeOD. This solvent has an intermediate polarity so it dissolves both forms of Thio and aggregation was much lower. Characterization was carried out from the chemical shifts in the ^{1}H NMR spectral data (Table 1). Fig. 4 shows the structure of each Thio species (the cationic form (**I**) and the free base form (**II**)); the characterization of **II** was performed at pH = 14 with addition of NaOD in order to obtain the complete conversion of **I** to **II**.

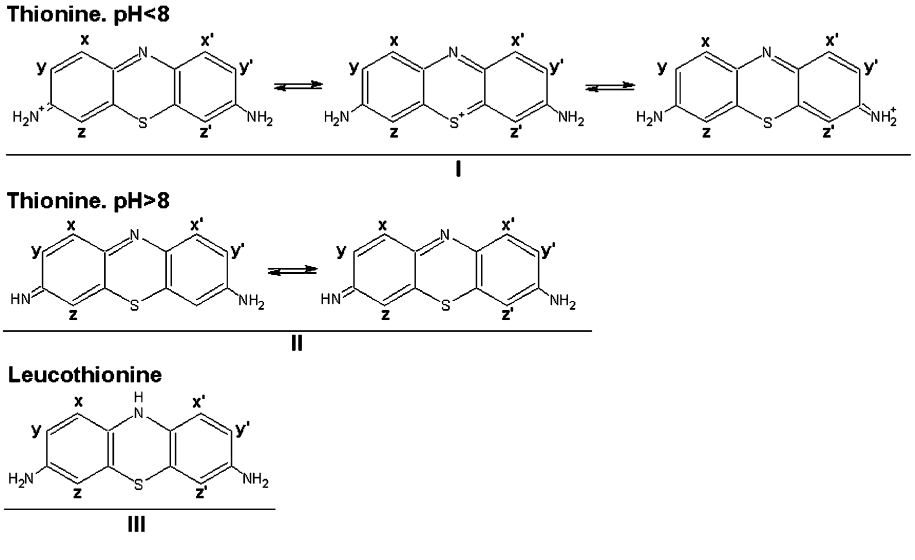

Fig. 4. Chemical structures of the thionine species characterized in different media.

The ^{1}H NMR spectrum of **I** exhibited only three signals corresponding to aromatic protons at δ 8.05 (d, $J = 9.5$ Hz), 7.34 (dd $J = 9.5$ and 2.2 Hz) and 7.22 (d, $J = 2.2$ Hz), assigned to H-xx′, H-yy′ and H-zz′, respectively. The ^{1}H NMR spectrum of **II** was closely related to **I**. The differences observed between the spectra of **I** and **II** were the highfield shifts for the three protons from δ 8.05 (H-xx′), δ 7.34 (H-yy′) and δ 7.22 (H-zz′) to δ 7.33, δ 6.87 and δ 6.70, respectively, indicating that the species **II** was shielded. These results support the lack of cationic nature for **II**.

The product of thionine photoinduced reduction, leucothionine (**III**), was obtained in deuterowater–methanol-d_4 (3:2) using Na_2SO_3 in excess to reduce Thio until the solution was colorless under illumination; the product was characterized by ^{1}H NMR spectroscopy. The ^{1}H NMR spectrum of **III** was similar to those of compound **II**, and showed three signals at $\delta = 7.29$ (d, $J = 8.8$ Hz), 6.72 (br d, $J = 8.8$ Hz) and 6.77 (br s) assigned to H-xx′, H-yy′ and H-zz′, respectively. The chemical shifts observed were consistent with the lack of conjugation between the phenyl rings.

4. Conclusions

Forms of thionine existing in different media were characterized using spectroscopic methods (UV–vis and ^{1}H NMR). At basic pH values the free imine form of the thionine dye is formed which is of a highly hydrophobic nature. Besides, the monomeric cationic thionine form can be photoreduced in both organic and aqueous solutions, while the free imine form did not show any photoactivity in those media.

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