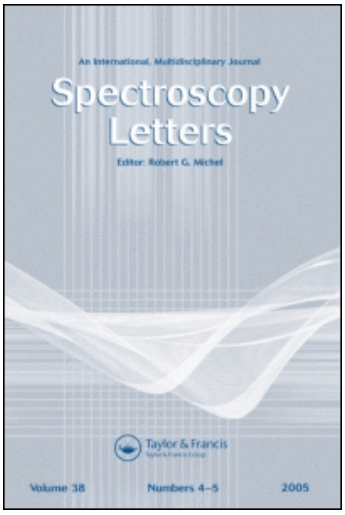

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Electronic absorption and fluorescence spectra of fluorescein in aprotic solvents

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Abstract

The absorption, excitation and fluorescence spectra of fluorescein in ethyl acetate and acetone were obtained. It was verified that the neutral lactonic form of fluorescein is present in dilute solutions of those organic solvents. However, in concentrated solutions it was observed a shift of the dissociation equilibrium to form the neutral zwitterionic form of the dye in both electronic ground and excited states.

I - Introduction

Fluorescein is an important xanthene dye with a large variety of technical applications due to its high fluorescence quantum yield. The photophysics of fluorescein is an active field of research because of the possibility of a number of ionic forms, such as dianion (pKa = 6.7), monoanion (pKa = 4.4), cation (pKa = 2.13) and neutral forms such as lactone, quinonoid and zwitterion[1]. Its photophysical and spectral characteristics have been studied in aqueous solutions as a function of concentration of the dye and it has been established that the bands for the monomeric forms are present at concentrations lower than 10^{-5} M[2,3].

They are also dependent on the viscosity, polarity and polarizability of the solvents, as well as on the pH of the aqueous solutions.

Although the different molecular forms of fluorescein have been reported and identified by optical spectroscopy using protic solvents, the photophysical properties of the neutral forms in aprotic solvents are not well known. For instance it is well known that the neutral zwitterionic form of this dye didn't show any fluorescence emission in aqueous solution due to a protolytic chemical reaction in the electronic excited state. In this work we present studies using optical spectroscopy (absorpion and emission) of fluorescein in the neutral forms (lactonic and zwitterionic) in aprotic solvents, such as ethyl acetate and acetone in both dilute and concentrated solutions. Its is also discussed the relationship between the dissociation equilibrium and the aggregation processes in concentrated solutions.

II - Experimental

Fluorescein (Merck) was purified by a process named lactonization, that consists of a recrystallization from a basic aqueous solution using hydrochloric acid[4]. Ethyl acetate and acetone were dried and distilled.

The solutions were prepared by dissolving the accurately weighted material in the above solvents and making successive dilutions. The acidic aqueous solutions was prepared using sulfuric acid. In order to obtain intermediate pH values it is used buffered solutions using monobasic sodium phosphate and dibasic sodium phosphate. A Digimed model DMPH2 pH-meter was used for pH-determinations of the aqueous solutions at room temperatures.

Electronic absorption spectra of fluorescein in the different solvents were recorded in a Varian-Carry 2300 spectrophotometer using a 10 mm optical pathlength cuvette. Fluorescence and excitation spectra were obtained using a Perkin Elmer model MFP-44B spectrofluorimeter with a 150 W xenon lamp and a Hamamatsu Corporation model R928 photomultiplier tube. All the measurement were done on a freshly prepared solution at room temperature.

III - Results and discussion

Electronic absorption or excitation spectra of fluorescein in different solvents are known to be influenced by the total concentration of the solute. In

dilute solution of ethyl acetate (10^{-5} M) the excitation spectrum is similar to the electronic absorption one and is characterized by a band with vibronic structure with a maximum at ~275 nm. This band is assigned to the absorption of the lactonic form[3,5] (figure 1.a). A broad and structureless band is observed in concentrated solution (10^{-3} M) (figure 1.b), with λ_{max} ~440 nm, similar to that presents in aqueous solution with pH ~2.0, which may be assigned to the neutral form of fluorescein with electronic conjugation on the xanthenic ring (figure 4.b).

Fluorescence spectrum of fluorescein in dilute solutions of ethyl acetate (λ_{exc} = 275 nm) is composed by a very weak and broad band centred at 410 nm (figure 1.c). However, in concentred solutions the fluorescence spectrum is composed by two broad bands, one most intense at 475 nm with a shoulder at 410 nm (figure 1.d).

The electronic absorption and the fluorescence spectra allow us to conclude that the lactonic form of this dye presents in dilute solution of the ethyl acetate may exist in this aprotic solvent in both electronic ground and excited states, similarly to that observed for rhodamine dyes[16].

The observed spectra of fluorescein in acetone solutions are quite similar to those in ethyl acetate ones: 1- in dilute solutions (10^{-5} M) both excitation and absorption spectra have a similar profile; 2- the fluorescence spectrum (excitation at 275 nm) in dilute solution exhibits a weak and broad band at 410 nm (figure 2.a); 3- in concentrated solutions ($8.4 \cdot 10^{-4}$ M) the electronic excitation and absorption spectra are also quite similar with a broad band at ~440 nm (figure 2.d); 4- fluorescence spectrum (excitation at 330 nm) of this sample shows two bands at ~475 nm with a shoulder at 410 nm (figure 2.c). Moreover the intensity of the fluorescence spectrum of the dye in dilute solution is strongly dependent on the excitation wavelength. The most intense spectrum is obtained with excitation wavelength at 330 nm, which is resonant with the solvent electronic absorption (figure 2.b). This result suggests that an efficient energy excitation transfer process is involved between the solvent and the dye molecules in the same sense as indicated for other systems like xanthenic dyes and enzimatically generated triplet acetone[7].

Intermolecular energy transfer process from enzyme generated triplet acetone to xanthene dyes (uranine, eosine and rose bengal)[7] may involve triplet-triplet transfer to generate an upper triplete state of the dye, with subsequent intersystem crossing to the fluorescent S_1 state. However, in the case of fluorescein in the lactonic form the intermolecular energy transfer

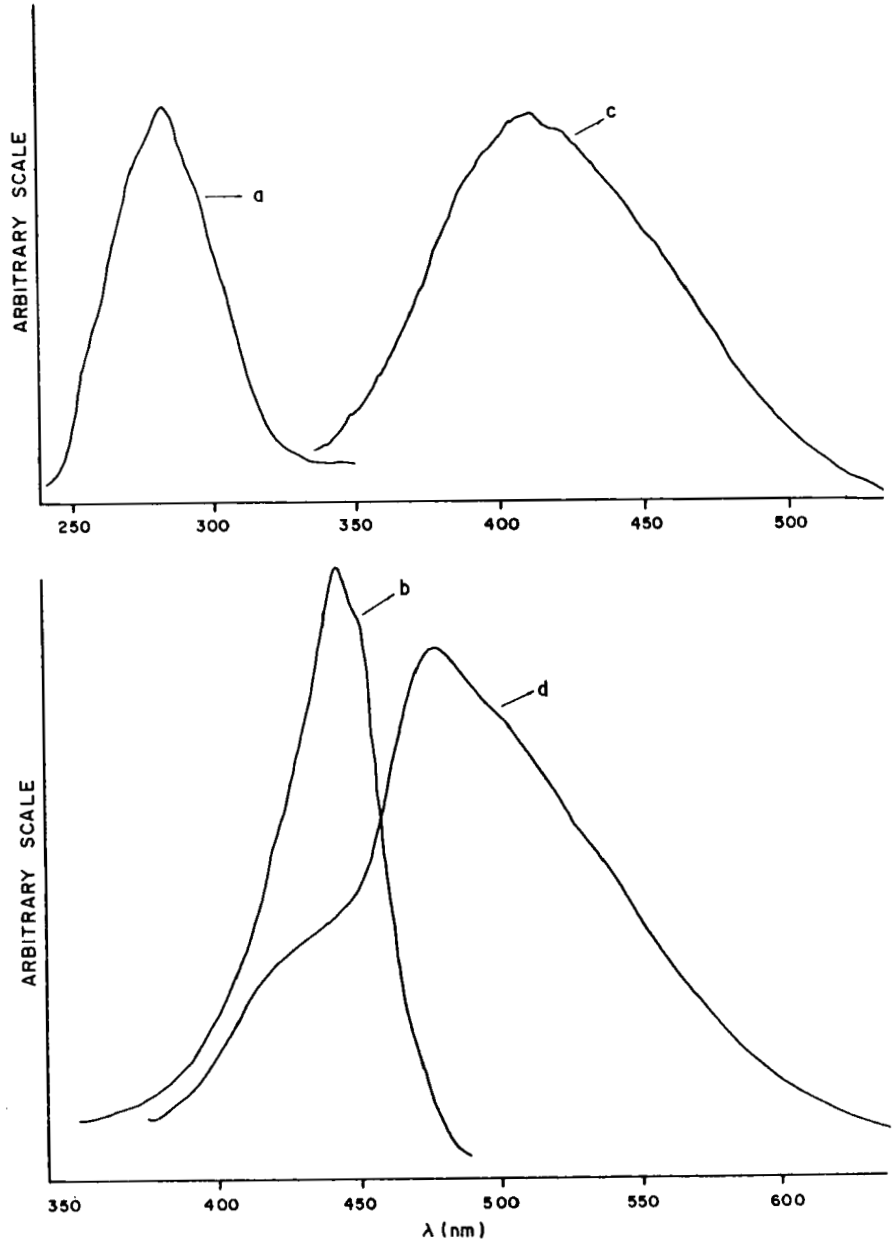

Figure 1 - Electronic excitation spectra of fluorescein in ethyl acetate: a- $1.0 \cdot 10^{-5}$ M (λ_{em} = 410 nm), b- $1.0 \cdot 10^{-3}$ M (λ_{em} = 475 nm); fluorescence spectra: c- $1.0 \cdot 10^{-5}$ M (λ_{exc} = 275 nm), d- $1.0 \cdot 10^{-3}$ M (λ_{exc} = 360 nm).

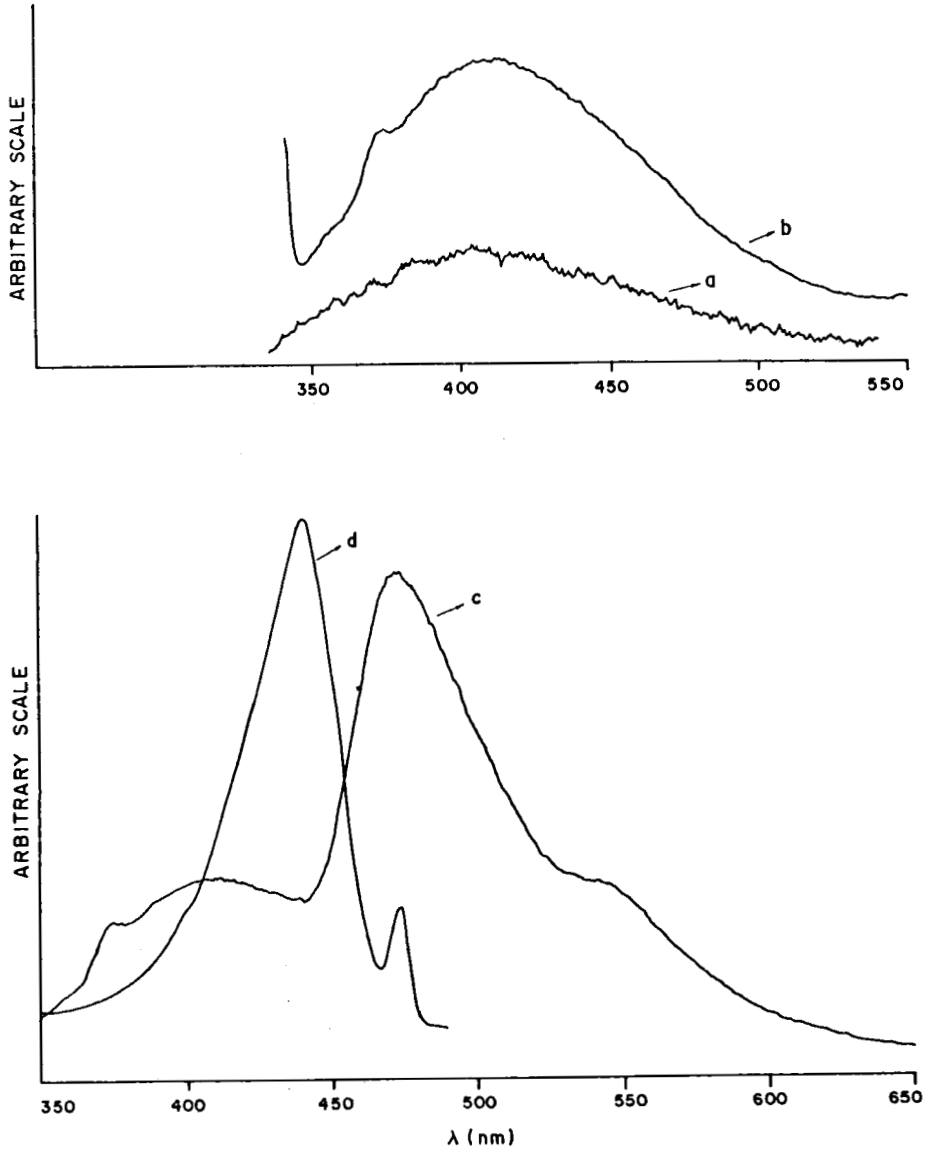

Figure 2 - Fluorescence spectra of fluorescein in acetone: a- $1.0 \cdot 10^{-5}$ M (λ_{exc} = 275 nm), b- $1.0 \cdot 10^{-5}$ M (λ_{exc} = 330 nm), c- $8.4 \cdot 10^{-4}$ M (λ_{exc} = 330 nm); d- electronic excitation spectra (λ_{em} = 475 nm), c = $8.4 \cdot 10^{-4}$ M.

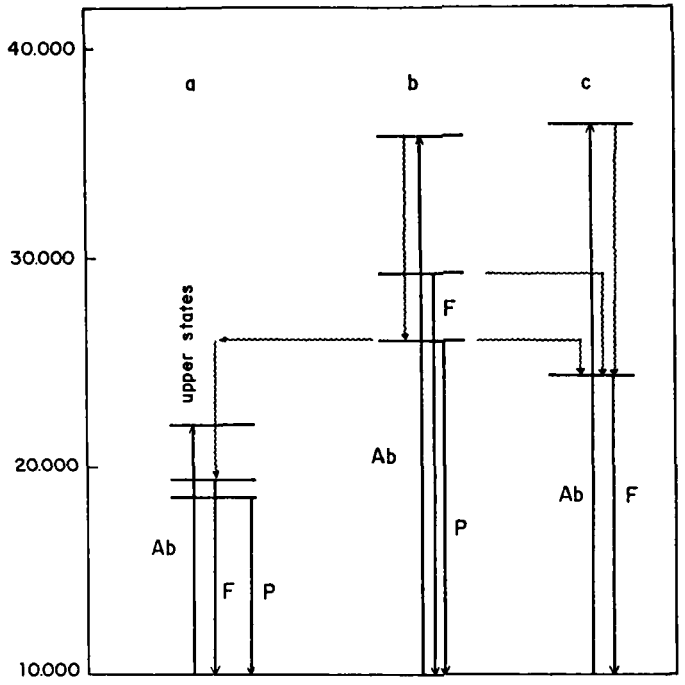

Figure 3 - Diagram of the energy levels of uranine (a), acetone (b) and fluorescein (c). Straight arrows indicate radiative transitions; wavy arrows indicate nonradiative transitions; Ab = absorption, F = fluorescence, P = phosphorescence.

process is almost resonant (figure 3). Consequently this process may be much more efficient.

In order to identify the forms responsible by the absorption and emission of the fluorescein in concentrated solutions of aprotic solvents, we obtained the absorption and fluorescence spectra in acidic and buffered aqueous solutions, particularly in the pH-range of $2.0<pH<4.3$[8,9]. In this range there is a dissociation equilibrium involving the neutral forms of the dye such as: lactone, zwitterion and quinonoid. It is also well known that in this pH-range the solubility of fluorescein in aqueous solution is very low[9].

It is observed that both cationic and zwitterionic forms of fluorescein present in acidic aqueous solutions exhibit an electronic absorption band with a

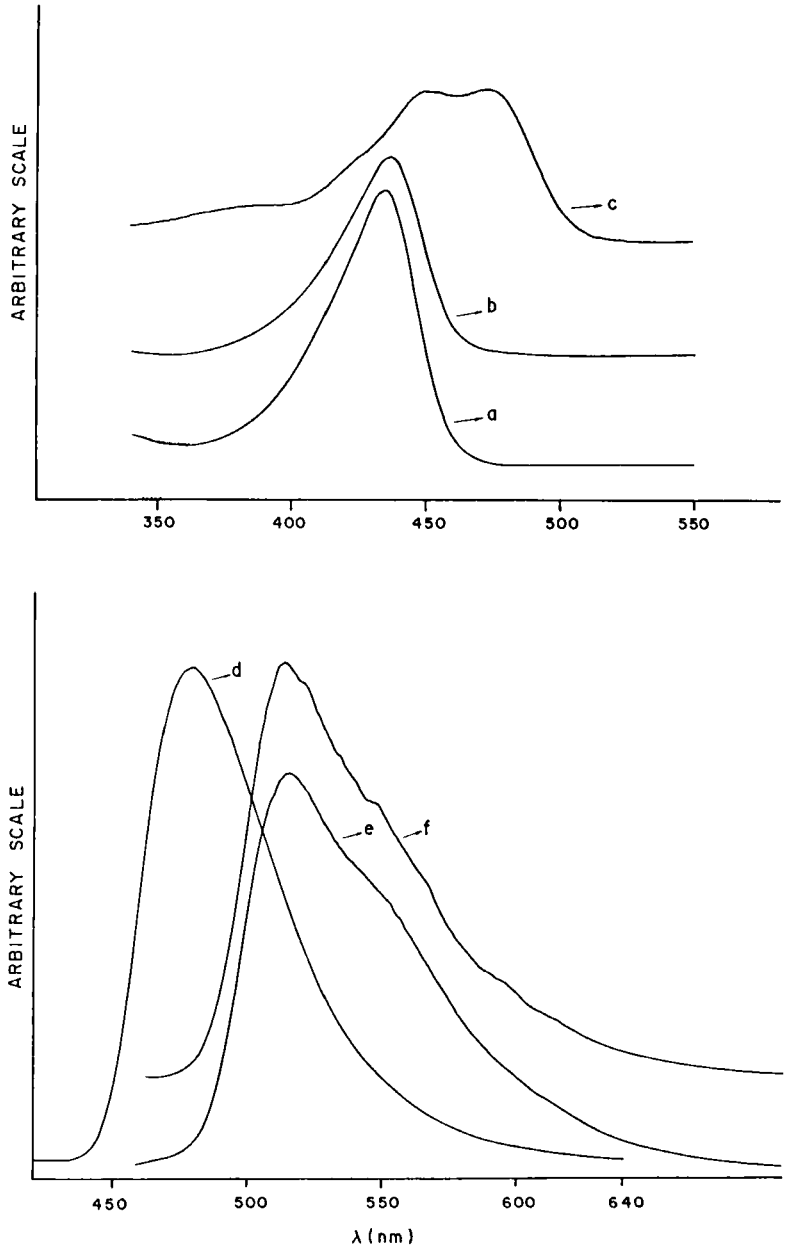

Figure 4 - Electronic absorption of fluorescein in aqueous solutions ($c = 8.3 \cdot 10^{-6}$ M): a- H_2SO_4 10 M, pH = 1.8, c- pH = 5.6; fluorescence spectra: d- H_2SO_4 10 M, e- pH = 1.8, f- pH = 5.6. (λ_{exc} = 360 nm).

maximum at ~436 nm. This band is assigned to the positively charged xanthene ring (figures 4.a, b)[3,5,8,9]. The fluorescence spectrum of the cationic form shows a band at 475 nm (figure 4.d) assigned to the chromophore positively charged. However, the zwitterionic form don't show any emission in protic solvent due to a protolytic chemical reaction in the electronic excited state resulting in a quinonoid form[3,8] (figure 4.e). This form has the same xanthenic group as the monoanionic form and shows a similar fluorescence emission (figure 4.f). This reaction can not occur in aprotic solvent and consequently we have assigned the electronic absorption and fluorescence bands observed at 444 nm and 475 nm, respectively, to the zwitterionic form of fluorescien. These assignments are supported by the following assumptions: 1- the zwitterionic form has a positive charge on the xanthenic ring and consequently its absorption and emission spectra should be the same as observed for the cationic form; 2- in aprotic solvents the electronic excited state reaction of the zwitterionic form involving a protolytic chemical reaction is not possible; 3- rhodamine dye in different aprotic solvents exhibited a double emission which were assigned to the lactonic and quinonoid forms[10,11]. This last form has a similar charge distribution of the zwitterionic form of fluorescein. 4- aggregation process of the lactonic form of fluorescein has not been observed in solutions, consequently an increase of the concentration of the dye in solution induces a shift of the dissociation equilibrium. This last assumption is supported by other observations: both dianionic and cationic forms of fluorescein undergoes to aggregation processes in more concentrated solutions while the increase of the concentration of its neutral forms induces a shift in the dissociation equilibrium. Moreover it has recently established that the solvent molecules have an important function in the estabilization of the dimeric structure of the dyes like fluorescein, rhodamine and rose bengal[10,12-15]. In these cases the solvents decrease the electrostatic repulsion between the monomer units and they control the kind of structure of the dimers (sandwich or linear association).

V - Conclusions

We have demonstrated that the fluorescein in the lactonic form is only present in dilute solutions of aprotic solvents. In more concentrated solutions it is observed a shift of the dissociation equilibrium and the ampho-ion form of fluorescein exhibits an electronic absorption band at ~440 nm.

We have also demonstrated that ampho-ion exhibits fluorescence emission in aprotic solvent, supporting the assumption that in protic solvent this form undergoes to a chemical reaction in the electronic excited state. Its electronic absorption and fluorescence spectra have a contourn similar to that observed for cationic form supporting the assumption that it has a positively charged xanthenic ring.

Finally this work indicates that it is not possible the aggregation process of the fluorescein in the neutral lactonic form in aprotic solvents probabily because the absence of attractive electrostatic interactions between the monomer units.

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