Ultraviolet/Visible Absorption, Luminescence, and X-ray Photoelectron Spectroscopic Studies of a Rhodamine Dye Covalently Bound to Microcrystalline Cellulose

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ABSTRACT: Rhodamine B isothiocyanate was adsorbed onto microcrystalline cellulose by two different methods: deposition from ethanolic and aqueous solutions followed by solvent evaporation and also from aqueous solutions in equilibrium with the powdered solid and following a dyeing protocol. After the above-mentioned samples were carefully washed, the fluorescence quantum yields (ϕ_F) determined were about 0.40 ± 0.03 and 0.28 ± 0.03 for ethanol and water, respectively, solvents that efficiently swell cellulose, when these two solvents were used for sample preparation with the first method, while for dyed samples ϕ_F is only 0.10 ± 0.05. These values for ϕ_F can be compared with 0.70 ± 0.03 obtained for rhodamine B entrapped in the polymer chains of microcrystalline cellulose. X-ray photoelectron spectroscopic studies present evidence for a smaller positive charge density on the nitrogen atom for dyed samples when compared with the adsorbed ones. This is compatible with nitrogen atoms, which do not participate in the conjugated system. These findings indicate that rhodamine B has different conformers in dyed samples as compared to adsorbed samples. In the former case, the chemical bond, anchoring the dye to microcrystalline cellulose, leads to nonplanar conformers with smaller ϕ_F and fluorescence lifetime (τ_F) values. In the latter case, planar conformers predominate, with the consequent increase of both lifetime and fluorescent quantum yield.

1. Introduction

In the past decade we have made some surface photochemical studies of several dyes, including rhodamines, adsorbed onto a scarcely used adsorbent: powdered microcrystalline cellulose.[1−6] From these studies, a large amount of information concerning room-temperature fluorescence and phosphorescence,[1−4] triplet−triplet energy transfer,[3] electron and hydrogen transfer,[1c,d,5a,b] and the nature of the adsorption process[5c−e] was obtained. All these processes are strongly dependent on the interaction of the probes with the matrix, which may provide a rigid environment strongly affecting the properties of the guest molecules.

Rhodamine B and related xanthene dyes have been extensively studied in solution.[7−11] Both molecular structure and solvent play a very important role in the nonradiative pathway of deactivation of the first excited state of these dyes.[7−11] The cationic forms of rhodamine B and rhodamine 101 in acidic ethanol present different fluorescent emission quantum yields: for the former, rhodamine $\phi_F = 0.49$ and this efficiency varies strongly with temperature, while for the latter ϕ_F is unitary and independent of temperature.[7,8] The amino groups in rhodamine B are fully ethylated, while in rhodamine 101 the amino groups are rigidly attached to the xanthene ring by methylenic bridges.

The precise interpretation of the mechanism of internal conversion appears not to be completely established: on the basis of rotation about the xanthene−amine bond, Drexhage proposed, in the 1970s,[7] an intramolecular rotation model, which was later disputed by other research groups. Rettig and co-workers proposed a twisted intramolecular charge-transfer mechanism (TICT, electron transfer from the amino group to the xanthene moiety, followed by rotation about the xanthene−amine bond), as the main mechanism for nonradiative deactivation.[9]

Chart 1 shows the most important resonance contributors of the acid form of rhodamine B isothiocyanate, which clearly illustrates that forms a and c are planar, while in form b the amino group is pyramidal. In an interesting series of papers on rhodamine dyes, Lopez Arbeloa et al.[10] and others[11] claimed that internal conversion may be associated with a change from planar (and fluorescent) structure to a pyramidal and nonfluorescent one upon absorption of a photon. As a result of this umbrella-like motion (ULM) the positive charge moves from the amino group to the xanthene group and the double bond character of the xanthene amino group is lost. The electron system is interrupted, and the amino group may rotate after excitation.

The picture described so far for rigid and nonrigid rhodamines applies essentially to solution, in either viscous or nonviscous systems.

In the solid state the photophysics of rhodamine B and rhodamine 101 may be markedly different from solution. As an example, rhodamine B adsorbed on glass or naphthalene exhibits a long lifetime of about 3.8 ns and deviations from monoexponential decay occur even at very low coverages[12] (τ_F of rhodamine B in ethanol is 2.85 ns and τ_F of rhodamine B in water is 1.63 ns, and they are strictly single exponential decays).

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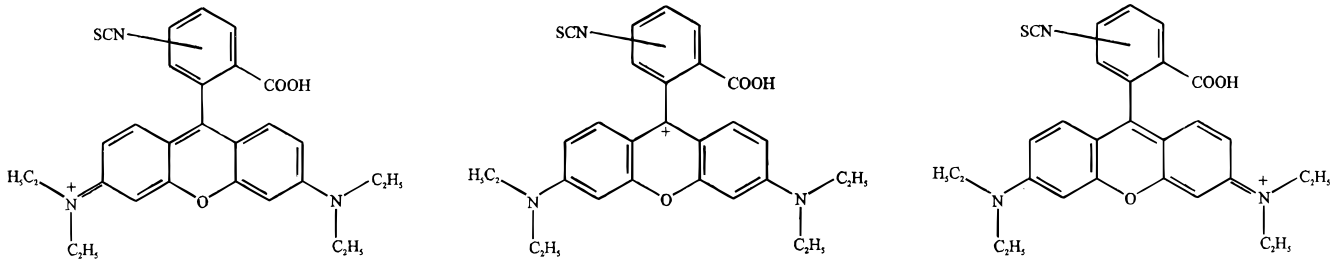
Chart 1



(a) **(b)** **(c)**

Rhodamine 101 also shows a monoexponential decay in ethanol (τ_F in ethanol is 4.3 ns and in water is 4.2 ns), while that adsorbed on glass with distorted adsorption sites shows a marked decrease in the lifetime.[12b] This fact is indicative of the formation of nonplanar conformers, as the one in Chart 1 structure b, responsible for the decrease of the lifetime of the excited singlet state due to enhanced internal conversion.[12b]

The most important distinguishing characteristic of reactive dyes is that they form covalent bonds with the substrate that is to be colored in the dyeing process. The dye molecule contains specific functional groups that can undergo addition or substitution reaction with the −OH, −SH, or $-NH_2$ groups that are present in the textile fibers.[13] According to the number of reactive systems they contain, reactive dyes can be classified as single-, double-, and multiple-anchor dyes. The most important application of reactive dyes is the dyeing and printing of cellulose fibers and cellulose fiber-based materials.[13,14]

Rhodamine B isothiocyanate is a reactive single-anchor dye that can easily be chemically bound to cellulose in the presence of a base.[14]

Adsorption of rhodamine B isothiocyanate onto microcrystalline cellulose can be done using solutions of the probe dissolved in polar protic solvents such as ethanol or water. When this solid substrate is added to such solutions, cellulose to cellulose hydrogen bonds are replaced by cellulose to solvent bonds and the matrix exhibits a certain degree of swelling. Cellulose swelling is highly dependent on the solvent used for sample preparation.[1,2] In the case of good swelling solvents, probes can penetrate into submicroscopic pores of the solid substrate and stay entrapped in the natural polymer cellulose chains after solvent removal.

X-ray photoelectron spectroscopy (XPS) is a powerful tool for surface characterization. The technique is strongly surface sensitive due to the photoelectron escape depth: only the first 10−20 atomic surface layers can be analyzed.[15] XPS is capable of providing information about elemental concentration by angle-resolved analysis only for nearly ideal surfaces: flat, smooth, nonporous, homogeneous in the plane of the surface, nonvolatile, conductive, and stable with respect to ultrahigh vacuum. Cellulose is far from fulfilling these criteria. However, despite these difficulties, a lot of interesting work with cellulose,[5e,15,16] cellulose-based materials[17] and other porous materials[18,19] has recently been reported.

Following previous studies of rhodamine dyes[1a,b] adsorbed onto microcrystalline cellulose,[6] in the present paper we report spectroscopic information regarding this dye covalently bound to the natural polymer. These two types of samples will be compared from the point of view of UV/vis ground-state diffuse reflectance measurements as well as some steady-state and transient luminescence studies, providing a close insight into the nature of the interactions between the cellulosic substrate and the probes.

With the use of XPS technique and more specifically through the nitrogen 1s photoelectron region study, conclusions can be drawn regarding the influence of the intermolecular interactions and reaction between the dye and the substrate concerning molecular planarity.

With these studies we hope to gain an insight into the nature of the interactions of rhodamine B with the polymer chains, namely establishing clear differences between physically and chemically adsorbed rhodamine B onto cellulose.

2. Experimental Section

2.1. Materials and Sample Preparation. Rhodamine B isothiocyanate (chloride) and rhodamine B (perchlorate) were purchased from Aldrich and Radiant Dyes Chemie, respectively, in the highest purity available. Ethanol was from Merck (Uvasol grade), and water was twice distilled and passed through a Millipore Milli-Q water purification system. Rhodamine B was a laser dye used without further purification after checking its purity by means of UV/vis absorption spectra and thin-layer chromatography. All these solvents were used as received, after checking their purity by UV and visible optical absorption spectrophotometry. In the case of rhodamine B isothiocyanate, which is sold as a mixture of isomers (different attachment of the isothiocyanate group to the carboxyphenyl group, as shown in Figure 1), thin-layer chromatography revealed the presence of impurities, probably resulting from decomposition of the isothiocyanate derivative of rhodamine B in the presence of humidity. Elemental analysis revealed that only about 35% of the product sold by the producer was the rhodamine B isothiocyanate. In industry, dyes used for dyeing fabrics are normally far from being pure. Since our intention was to produce dyed samples following an industrial protocol, where the final step is to carefully wash up the samples, removing all the dye that is not chemically adsorbed, we opted to use the product as supplied by the manufacturer.

Microcrystalline cellulose[6] (Fluka DSO) with 50 μm average particle size was dried under vacuum (ca. 10^{-3} mbar) at 60 °C for at least 24 h before sample preparation.

Two main types of samples were prepared.

Type I samples or *deposited samples*, where rhodamine B isothiocyanate is adsorbed by slow solvent evaporation. In this case, samples were prepared as described in refs 1a−c except for the final solvent removal step, which was performed in an acrylic chamber with two electrically heated shelves (Heto, models FD 1.0-110 and FC-2R/H) with temperature control (40 ± 1 °C) and reduced pressure (ca. 10^{-3} mbar, 12 h). Two different solvents were used for adsorption, ethanol and water, producing samples here called ethanolic samples and aqueous samples, respectively.

For comparison purposes, type I samples were also made using rhodamine B and ethanol as solvent for sample preparation.

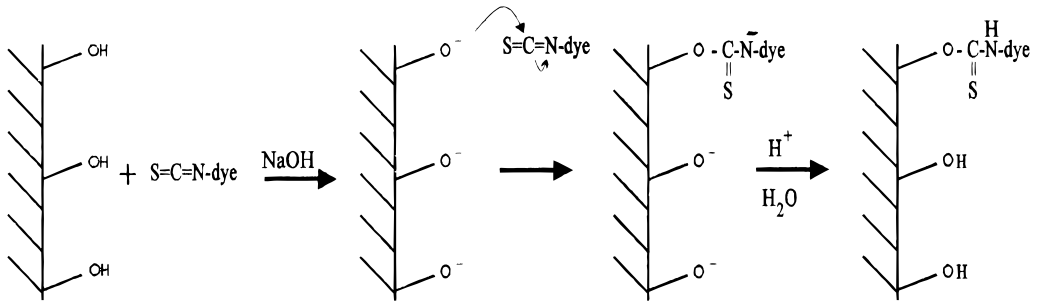


Figure 1. Dyeing reaction of rhodamine B isothiocyanate with cellulose, which results in a covalent bond formation anchoring the dye to the natural fiber.

Type II samples or *dyed samples*, where the powdered cellulose is in contact with an aqueous solution of a reactive dye (rhodamine B isothiocyanate) until all the reactive dye bonds to cellulose. The dyeing procedure used in this work was adapted from an industrial recommended dyeing methodology, called the isothermal-dispensing method, suitable for cellulose.[20] Dyeing was made at about 70 °C in a basic solution. NaOH and CO_3Na were used so that the hydroxyl groups of cellulose could lose a proton and in this way become a nucleophilic agent reacting with the isothiocyanate group of the reactive dye. A covalent bond is therefore formed between cellulose and rhodamine, according to Figure 1.[14] After water was added and after neutralization, the hydroxyl groups are again protonated, as well as the nitrogen atom.

At this stage, solvent was removed by filtration. The final evaporation of solvent and sample drying was done as described for type I samples.

Both type I and type II samples were repeatedly washed with water until no significative amount of dye was found in the washing water. The use of ground-state diffuse reflectance (GSDR) absorption spectrophotometry allowed us to evaluate the amount of dye that stayed adsorbed or bound to cellulose.

2.2. Ground-State Absorption Spectra in the UV/Vis Regions, IR Spectra, and Steady-State Emission Experiments. Ground-state absorption studies of rhodamine B isothiocyanate and rhodamine B adsorbed or covalently bound to microcrystalline cellulose were performed using an OLIS 14 UV/vis/NIR spectrophotometer with a diffuse reflectance attachment. The integrating sphere is 90 mm in diameter and internally coated with a standard white coating. The standard apparatus was modified to include the possibility of using short-wave-pass filters (Corion 600-S), which excludes the two rhodamines luminescence from reaching the detector (Hamamatsu, Model R955).

Further experimental details and a description of the system calibration used to obtain accurate reflectance measurements are given in refs 1 and 2.

Solution absorption measurements were made using the same apparatus in the normal transmission mode.

Corrected steady-state fluorescence and phosphorescence emission and excitation spectra of the rhodamines under study, adsorbed or covalently bound to microcrystalline cellulose, were obtained using a homemade fluorometer previously described.[21]

Infrared spectra were performed with a Mattson 5000 FTIR spectrometer. All samples were diluted with KBr supplied from Aldrich (FTIR grade) in about 2% w/w and ground to a finely divided powder (except for crystals of rhodamine B isothiocyanate). In this case samples were prepared by solvent evaporation of an ethanolic solution of the dye cast over a sodium chloride disk. Spectra were recorded at 4 cm^{-1} resolution in the 400−4000 cm^{-1} range and were obtained as a ratio of 16 scans to the background signal (same number of scans, pure KBr). No baseline corrections were made.

2.3. Subnanosecond Fluorescence Lifetime Measurements. Subnanosecond lifetime measurements of rhodamine B isothiocyanate adsorbed or covalently bound to microcrystalline cellulose were made using a fluorescence microscope described in detail in ref 22b. Dye laser excitation pulses were of approximately 10 ps half-width. The excitation wavelength used in this work was 580 nm, and the dye laser pulse repetition rate was 0.8 kHz. In some cases, band-pass filters were used to reduce the fluorescence emission band that reaches the detector and neutral density filters to reduce laser energy. Data were recorded every 250 ps after excitation, using a 500 ps time gate.

2.4. XPS experiments. For X-ray photoelectron spectroscopy (XPS) studies, samples of dry cellulose with adsorbed dye were molded as for IR experiments, without any solid solvent, and held to the sample holder stub with a tungsten spring. Samples were analyzed by X-ray photoelectron spectroscopy (XPS) using an XSAM800 (KRATOS) X-ray spectrometer operated in the fixed analyzer transmission (FAT) mode, with a pass energy of 20 eV and the nonmonochromatized Mg Kα X radiation ($h\nu = 1253.7$ eV). Typical operating parameters were 13 kV and 10 mA. Samples were analyzed in ultrahigh vacuum (UHV), and the typical base pressure in the analysis chamber was in the range of 10^{-7} Pa. All sample transfers were made in air. Samples were analyzed at room temperature, at a takeoff angle (TOA) of 90°. Spectra were collected and stored in 200 channels with a step of 0.1 eV using a Sun SPARC Station 4 and with Vision software (Kratos). The curve fitting for component peaks was carried out with a nonlinear least-squares algorithm using a mixture of Gaussian and Lorentzian peak shapes.

Charge shifts due to the insulating character of samples were corrected through the C 1s cellulose component centered at 286.73 eV.[23]

3. Results and Discussion

3.1. Ground-State Absorption Spectra of Rhodamine B Isothiocyanate Covalently Bound to Microcrystalline Cellulose. Figure 2a shows the spectral reflectance (R) versus wavelength for rhodamine B isothiocyanate adsorbed onto microcrystalline cellulose from water (type I samples, after washing), while Figure 2b shows similar curves for dyed samples. In both cases, solutions of different concentrations were used for sample preparation, thus enabling us to compare different loadings of the dye on cellulose. The curves represent the percentage of reflectance as a function of wavelength in the range 420−620 nm. For shorter wavelengths (350 nm) the absorption of the dye is masked by the intrinsic absorption of microcrystalline cellulose.[1,2] It is interesting to note that similar concentrations provide quite different reflectance spectra, and absorption seems to be larger in deposited samples as compared to dyed samples.

The remission function $F(R)$ is obtained by calculating the Kubelka−Munk function for optically thick samples, i.e., those where any further increase in thickness does not affect the experimentally determined reflectance R.[1,24]

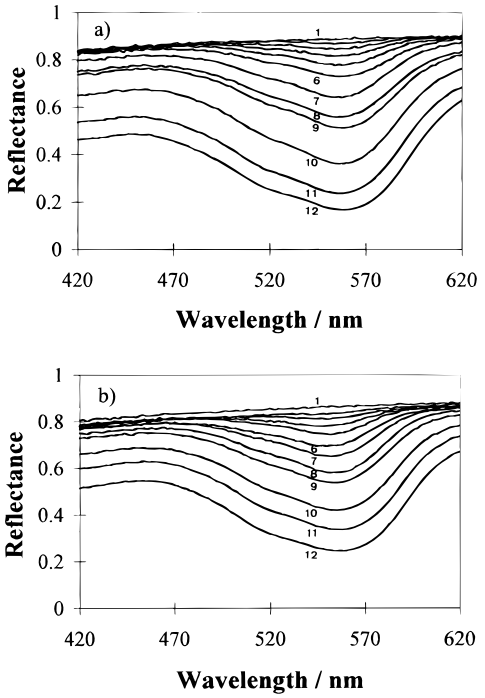


Figure 2. Reflectance spectra of rhodamine B isothiocyanate adsorbed onto microcrystalline cellulose. (a) "Type I" samples from aqueous solutions. Samples were repeatedly washed with water after the initial solvent evaporation. Curve 1 is the blank. Other curves: (2) 0.0015 μmol g^{-1}; (3) 0.003 μmol g^{-1}; (4) 0.0075 μmol g^{-1}; (5) 0.015 μmol g^{-1}; (6) 0.030 μmol g^{-1}; (7) 0.075 μmol g^{-1}; (8) 0.15 μmol g^{-1}; (9) 0.30 μmol g^{-1}; (10) 0.75 μmol g^{-1}; (11) 1.5 μmol g^{-1}; (12) 3.0 μmol g^{-1}. (b) "Type II" or dyed samples, from aqueous solutions. Samples were repeatedly washed with water after dyeing. Curve 1 is the blank. Others curves: (2) 0.0018 μmol g^{-1}; (3) 0.0035 μmol g^{-1}; (4) 0.0088 μmol g^{-1}; (5) 0.018 μmol g^{-1}; (6) 0.035 μmol g^{-1}; (7) 0.088 μmol g^{-1}; (8) 0.18 μmol g^{-1}; (9) 0.35 μmol g^{-1}; (10) 0.88 μmol g^{-1}; (11) 1.8 μmol g^{-1}; (12) 3.5 μmol g^{-1}.

$$F(R) = (1 - R)^2/(2R) = K/S \qquad (1)$$

K and S are the absorption and scattering coefficients with dimensions (distance)$^{-1}$. For an ideal diffuser, where the radiation has the same intensity in all directions, $K = 2\epsilon C$ (ϵ is the Naperian absorption coefficient, C is the concentration).[24] Since the substrate usually absorbs at the excitation wavelength λ_e,

$$F(R)_{probe} = F(R)_{total} - F(R)_{cell} = \sum_i 2\epsilon_i C_i/S \qquad (2)$$

where $F(R)_{cell}$ is the blank obtained with a cell containing only microcrystalline cellulose. This equation predicts a linear relation for the remission function of the probe as a function of concentration (for a constant scattering coefficient) whenever the probe is in the form of a monomer.

A comparison of the remission function spectra obtained before and after washing allowed us to estimate that the amount of rhodamine B isothiocyanate that remained attached to microcrystalline cellulose for type I and type II samples was about 30% and 35%, respectively. In the case of rhodamine B adsorbed onto microcrystalline cellulose, the washing procedure is much more efficient and only about 5% stayed physically adsorbed onto the substrate. This is an interesting result which shows that, even in the case where rhodamine B isothiocyanate is simply deposited onto microcrystalline cellulose by evaporation of the aqueous solvent, the reactive dye stayed on the substrate, thus pointing to some kind of bonding formation, even in this case.

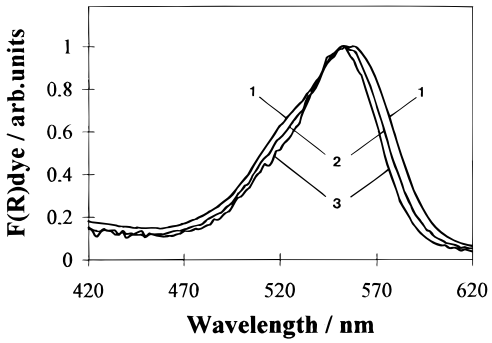


Figure 3. Remission function values for rhodamine B isothiocyanate covalently bound to microcrystalline cellulose, after dyeing samples were washed with water. Curves are normalized to the maximum of the remission function. Curve 1 is 3.5 μmol g^{-1}. Curve 2 is 0.35 μmol g^{-1}. Curve 3 is 0.035 μmol g^{-1}.

Figure 3 shows the remission function of dyed samples after washing, for different loadings (about 0.035, 0.35 and 3.5 μmol of dye/g of cellulose). All spectra are normalized to the maximum absorption.

There is a clear concentration dependence in what concerns the shape of the spectra: higher loadings seem to provide broader absorption bands although all samples were repeatedly washed and dye ground-state aggregation (for instance, dimer formation) becomes quite improbable.

Figure 4 compares the remission function of type II and type I washed samples, with the solution absorption of monomers of rhodamine B isothiocyanate, all spectra being normalized to the maximum. Figure 4a is for 0.035 μmol g^{-1} and Figure 4b is for 3.5 μmol g^{-1} loadings.

Type II samples of rhodamine B isothiocyanate are bathochromically shifted when compared with solution samples of the monomers of the same dye. However, when compared with type I samples, a clear hypsochromic shift is detected. Physically adsorbed rhodamine B entrapped within cellulosic fibers[25] also exhibits a red shift.

Unfortunately, IR spectra did not provide any useful information. Rhodamine B isothiocyanate crystals exhibit clear absorption bands of the isothiocyanate group at 752 cm^{-1} and in the range 2030−2150 cm^{-1} . Microcrystalline cellulose also gave a nice IR absorption spectrum, as described in the literature.[5c,6b] However, in the concentration range of the dye under study, bound or physically adsorbed to cellulose, no measurable absorption signal was detected in every case. Even in the case where higher concentrations were used (1.0 mmol of dye/g of cellulose), no detectable differences were observed when we compared pure and dyed cellulose.

3.2. Luminescence Studies of Rhodamine B Adsorbed and Covalently Bound to Microcrystalline Cellulose. We recently reported fluorescence emission studies of rhodamines[1a,b] and other dyes[1−5] at room temperature when these dyes were adsorbed onto microcrystalline cellulose.[1b] In the rhodamine case no significative phosphorescence was detected for any of the samples at room temperature when exciting at 337 nm with a nitrogen laser, as compared to fluorescence emission. Diffuse reflectance laser flash photolysis

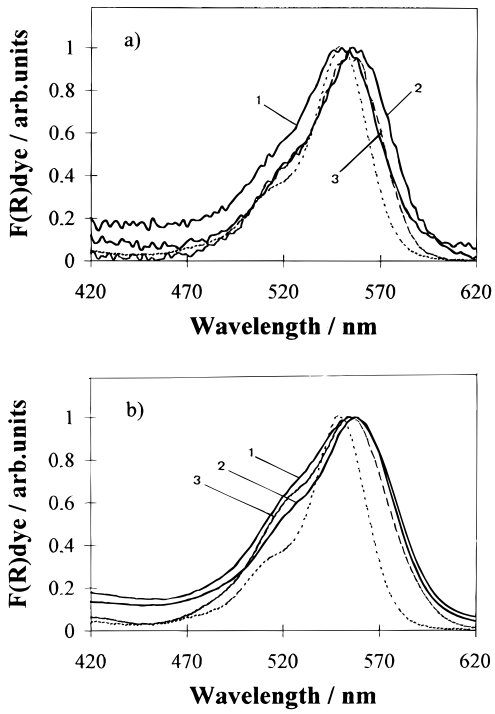


Figure 4. (a) Remission function values of rhodamine B isothiocyanate onto microcrystalline cellulose, normalized to the maximum of the absorption of the dye. Curve 1 is a type II sample with 0.035 μmol g^{-1}. Curve 2 is a type I sample with 0.030 μmol g^{-1}. Curve 3 is a 0.035 μmol g^{-1} rhodamine B physically adsorbed sample. The dashed curve is a diluted aqueous solution of the dye containing only monomers. (b) Remission function values of rhodamine B isothiocyanate onto microcrystalline cellulose and normalized to the maximum of the absorption of the dye. Curve 1 is a type II sample with 3.5 μmol g^{-1}. Curve 2 is a type I sample with 3.0 μmol g^{-1}. Curve 3 is a 3.5 μmol g^{-1} rhodamine B physically adsorbed sample. The dashed curve is a diluted aqueous solution of the dye containing only monomers.

studies for type I and type II washed samples showed no triplet−triplet absorption in the 350−900 nm region. This result indicates that neither triplet state nor radical anions or cations are formed after laser excitation at 532 nm when these two rhodamines are adsorbed onto microcrystalline cellulose.[25]

According to eq 3

$$I_F = GI_o(1 - R)f_{dye}\phi_F \quad (3)$$

a nice correlation of fluorescence emission intensity (I_F), measured as the total area under the emission spectra as a function of the light absorbed by the dye at the excitation wavelength, $(1 - R)f_{dye}$ is expected and was indeed found whenever the luminescent probes exist exclusively in the form of monomers. In eq 3, G is a geometrical factor, I_o is the excitation intensity at the excitation wavelength, R is the reflectance measured at the excitation wavelength, f_{dye} is the fraction of the excitation light absorbed by the dye at the excitation wavelength, and ϕ_F is the fluorescence quantum yield. Alternatively, I_F versus the square root of the concentration of the dye is also linear for small fractions of absorbed light. Deviations from linearity may occur for higher loadings due in many cases to aggregate formation, which are usually not fluorescent.[2a,b] Dimer emission was also reported in one case.[3a,4b]

Figure 5 shows the variation of intensity of fluorescence of rhodamine B isothiocyanate for type I and type II samples as a function of $(1 - R)f_{dye}$ for the dye adsorbed onto microcrystalline cellulose from ethanol and also from water. Rhodamine B type I samples from ethanol are also included for comparison purposes (curve 4).

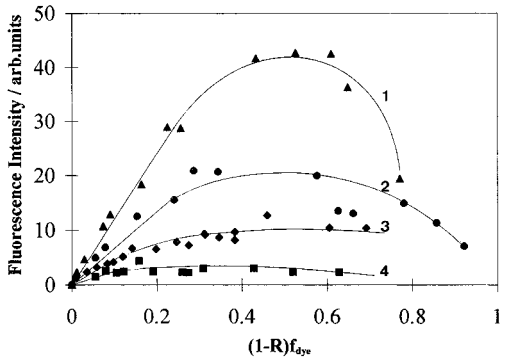


Figure 5. Variation of the intensity of fluorescence of rhodamine B and rhodamine B isothiocyanate adsorbed onto microcrystalline cellulose (steady state) measured as the total area under the corrected emission spectrum I_F as a function of $(1 - R)f_{dye}$: (curve 1) type I samples of rhodamine B prepared from ethanolic solutions; (curve 2) type I samples of the reactive dye prepared from ethanol; (curve 3) type I samples of the reactive dye prepared from water; (curve 4) type II or dyed samples. Samples from curves 3 and 4 were repeatedly washed after the initial solvent evaporation.

As reported before,[1a] fluorescence quantum yields of probes adsorbed onto powdered solids can be determined by the use of eq 4

$$\phi_F{}^u = \phi_F{}^s I_F{}^u(\lambda_e)(1 - R_s{}^{\lambda_e})f^s I_o{}^s(\lambda_e)/I_F{}^s(\lambda_e)(1 - R_u{}^{\lambda_e})f^u I_o{}^u(\lambda_e) \quad (4)$$

where the superscripts u and s refer to the unknown and standard samples.

$I_o{}^s(\lambda_e)/I_o{}^u(\lambda_e)$ can be easily obtained provided the energy profile of the system is accurately determined.[21] Rhodamine 101 was used as a reference compound ($\phi_F{}^s = 1.0$). The reasons for that were previously reported in refs 1a,b.

It is important to emphasize several important features: first, the initial slope (from the initial linear region) from type II samples after washing is the smallest one ($\phi_F = 0.10 \pm 0.05$), as is clearly shown in Figure 5, curve 4. The fluorescence emission quantum yield for type I samples prepared with water (curve 3, $\phi_F = 0.28 \pm 0.03$) is smaller than for ethanol samples (curve 2, $\phi_F = 0.40 \pm 0.03$).

Type I samples of rhodamine B from ethanol, where the dye is entrapped within the polymer chains,[1a,b] and in this way only physically adsorbed, are the ones that present the largest fluorescence quantum yield ($\phi_F = 0.70 \pm 0.03$).

These findings are once more consistent with the previously described picture: the use of ethanol, a polar and protic solvent, as a swelling agent for microcrystalline cellulose allowed us to entrap rhodamine B within the polymer chains, providing a rigid environment capable of causing enhanced fluorescence from this dye when comparing with the solution case at room temperature.[7,8]

The use of ethanol as solvent for the preparation of type I samples with the reactive rhodamine provides firmly bound dye instead of the simply physically adsorbed one; as we wrote before, in this case, washing

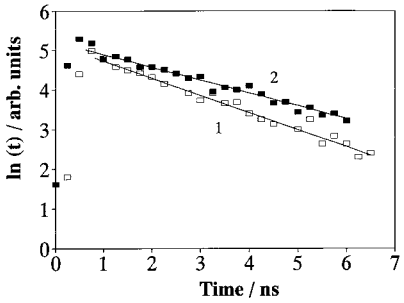


Figure 6. Fluorescence decay curves of rhodamine B isothiocyanate onto microcrystalline cellulose: (curve 1) type II samples, 0.35 mol g^{-1}; (curve 2) type I samples prepared from ethanol, 0.08 mol g^{-1}. All samples were repeatedly washed with water. The excitation wavelength was 580 nm.

Table 1. Lifetimes of Rhodamine B Isothiocyanate[a]

Covalently Bound to Microcrystalline Cellulose by the Use of an Industrial Dyeing Protocol				
conc/μmol g^{-1}	0.08	0.35	1.8	3.5
τ_F/ns	2.50	2.37	2.40	2.62
Adsorbed onto Microcrystalline Cellulose from Ethanol after Solvent Evaporation				
conc/μmol g^{-1}	0.08	0.75		
τ_F/ns	3.20	3.15		

[a] Both samples were washed repeatedly with water.

does not remove the dye as in physiadsorbed samples. The quantum yield of fluorescence is larger than in dyed samples but still smaller than rhodamine B simply entrapped within the polymer chains. With the use of water, a smaller cellulosic surface area is available for entrapment, possibly as a consequence of the aggregation of the reactive dye, which increases in water relative to ethanol, as many dyes do, due to the relative increase in hydrophobicity of the dye going from one solvent to the other.[7a]

Dyed and washed samples (curve 4 in Figure 5), exhibit the lowest fluorescence quantum yield.

Subnanosecond lifetime determination provided fluorescence decays that are presented in Figure 6 and are clearly nonexponential. This is not unexpected at all. In another case[4a] we detected deviations from a monoexponential decay due to a nonhomogeneous distribution of the adsorption sites and different conformational restrictions. In this case, and in contrast with the previous reported data analysis (see ref 4a, where we used dispersive kinetics and obtained an average rate constant for the decay, as well as a width of distribution), since we have curves with about 25 points per decay, we felt we should simply estimate the lifetime by the use of a very simple approach, a single-exponential fit. The result of these data treatment is presented in Table 1: type II samples have a lifetime of $\tau_F = 2.5 \pm 0.10$ ns and type I samples $\tau_F = 3.2 \pm 0.10$ ns, independently of the loading of the dye (see Figure 6). These decay times can be compared with the value of 3.73 ± 0.12 ns obtained for rhodamine B entrapped within cellulose.[26]

All the results presented in sections 3.1 and 3.2 point to the formation of rhodamine B isothiocyanate conformers: for type II samples, the higher the loading of the reactive dye, the larger is the formation of dye−cellulose covalent bonds, which promote nonplanar conformers of the dye.

When the reactive dye is added to the slurry cellulose/aqueous solution, the best surfacial pores for adsorption are occupied and the reaction cellulose−reactive dye occurs. As the concentration of reactive dye is increased, worse places for anchoring become occupied and more and more conformers become nonplanar, with the consequent decrease of conjugation, which leads to the hypsochromic effect in the remission function. This effect is particularly important because the dyeing protocol accelerates the kinetics of the covalent bond formation process. The final aqueous washing procedure removes all or at least the majority of the molecules that are simply physically adsorbed to microcrystalline cellulose, as the washing of rhodamine B samples physically adsorbed clearly proved.

For type I samples with rhodamine B isothiocyanate prepared from water at room temperature, covalent bond formation also occurs, since after washing all the reactive dye stays attached to the cellulosic fiber. However, this reaction is a slow one, and molecules have time enough to "select" an optimum microscopic pore to become adsorbed. Only then may the reaction with the hydroxyl groups of cellulose occur. In this case conjugation is still high, and as a consequence, this absorption curve is similar to the case of the physically adsorbed dye, entrapped within the natural polymer chains, although the broadening of the spectra is somehow larger due to the increase in the number of conformers which were formed.

All the spectroscopic evidence presented until here (ground-state absorption data), as well as both fluorescence quantum yields and lifetimes variation from simple physically adsorbed samples to dyed samples, relates to the chemical bond formation and the appearance of nonplanar conformers and supports the above-mentioned assumption. As we will mention in paragraph 3.3, XPS experiments will further support this interpretation of the GSDR absorption and fluorescence emission data.

3.3. XPS studies. The XPS studies of type I and type II rhodamine samples were mainly centered on the nitrogen region, as nitrogen is exclusively in the dye molecule, whereas carbon and oxygen are also important constituents of the substrate (carbon is even the major one). On the other hand, since nitrogen electrons can be involved in the delocalized π system, nitrogen is a good element to provide information on conformational changes or intermolecular interactions which may contribute to modify the nitrogen density charge. As an example, a large planarity corresponds to a large participation of nitrogen atoms in the delocalized π system. A high positive charge is, in this case, located at each of the nitrogen atoms bound to the xanthenic group, and the binding energy increases; they are both equivalent and, therefore, the N 1s XPS peak should be sharp, presenting a single component. Inversely, if methyl groups are out of the xanthenic plane, the electronic density around those nitrogen atoms will increase and the N 1s binding energy decreases; in addition, they may become nonequivalent and the N 1s XPS peak broadens.

Sulfur atoms are also present exclusively in the dye molecule but they are not so simple to analyze as nitrogen, since their concentration is low, one atom per molecule (vs three of nitrogen) and also because they are far from the xanthenic group. Moreover, since the isothiocyanate group (SCN) plays the role of anchor, it will be essentially buried in the substrate; therefore its contribution to the whole signal is weak. As a consequence, the information extracted from the N 1s region,

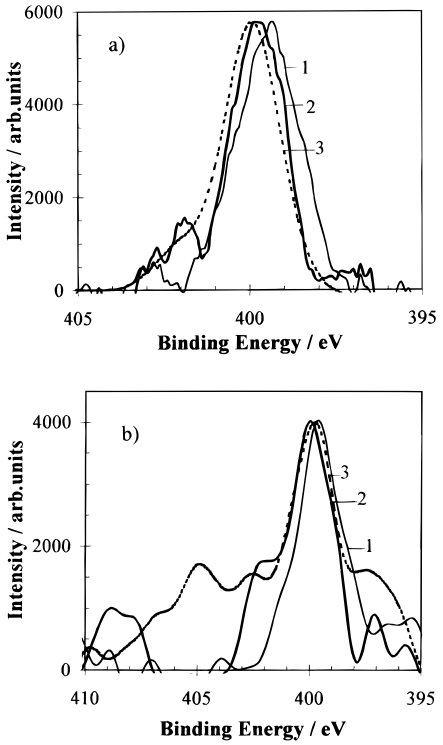


Figure 7. (a) XPS spectra for the N 1s region of samples with loadings of 3.5 μmol of rhodamine B isothiocyanate per gram of cellulose. Curve 1 is a type II sample repeatedly washed with water. Curve 2 is a type I sample from aqueous solution after washing with water. Curve 3 is for rhodamine B physically adsorbed from ethanolic solutions and subsequent removal of solvent by evaporation. (b) Same data as for part a but now for 0.35 μmol g^{-1}.

Table 2. Curve Fitting of the XPS N 1s Region for Three Samples of Microcrystalline Cellulose Loaded with about 3.5 μmol g^{-1} of Rhodamine B Isothiocyanate and Rhodamine B

	BE/eV	%	FWHM/eV
chemically adsorbed (dyed)	398.8	41.5	1.3
	399.9	43.9	1.3
	401.6	14.6	1.3
physically adsorbed from an aqueous solution after washing	399.3	30.4	1.1
	400.1	55.2	1.3
	402.1	14.4	1.2
rhodamine B physically adsorbed from an ethanolic solution	399.2	34.6	1.3
	400.1	49.2	1.3
	401.9	16.2	1.3

concerns mainly the two nitrogen atoms belonging to the xanthenic part of the molecule.

Figure 7a shows the XPS spectra for the N 1s region of type I and type II samples of rhodamine isothiocyanate as well as rhodamine B. Clear differences between them are observed: curve 1 is a type II sample (chemically bound dye) and is centered around 399.5 eV; curves 2 and 3 show increasing shifts toward larger binding energies. They correspond respectively to a type I sample (from aqueous solutions), subsequently washed with water, and to rhodamine B type I sample from an ethanolic solution. The relative composition of each peak is given in Table 2. The constraint imposed in the curve fitting regards the peak width, which was kept similar for all the peaks and in the range 1.1−1.3 eV, except for the spectrum that presents large "wings".

These results show that the sample with the highest electronic density around nitrogen atoms is the dyed one. This is indicative of a poor participation of nitrogen

Table 3. Curve Fitting of the XPS N 1s Region for Three Samples of Microcrystalline Cellulose Loaded with about 0.35 μmol g^{-1} of Rhodamine B Isothiocyanate and 1.0 μmol g^{-1} of Rhodamine B

	BE/eV	%	FWHM/eV
chemically adsorbed (dyed)	398.6	29.2	1.3
	399.8	50.6	1.1
	401.0	20.2	1.3
physically adsorbed from an aqueous solution after washing	399.2	22.0	1.2
	400.3	49.4	1.3
	402.3	28.6	1.3
	397.4	27.6	3
rhodamine B physically adsorbed from an ethanolic solution	399.4	15.8	1.1
	400.4	15.0	1.3
	402.2	5.1	1.3
	404.6	36.5	4.9

atoms in the delocalization, i.e., an increase in sp_3 character when compared to the physical adsorption case (structure b, Chart 1). This again is consistent with the previous hypothesis that the chemical reaction of the isothiocyanate group occurs with cellulose deep sites; since that molecular end group stays constrained by the chemical bond, geometrical hindrance to the planarity of the whole molecule exists, putting the positive charge in the xanthene moiety of the molecule instead of the nitrogen atoms.

The behavior of the sample where dye is physically adsorbed from an aqueous solution and subsequently washed is also very interesting: it presents a shift toward higher binding energies (BE). These data are consistent with the previous data in sections 3.1 and 3.2: in aqueous solution and in the absence of dyeing conditions, dye molecules adsorb on the most favorable sites, being free to acquire a planar configuration. Nitrogen atoms (or, at least, one of them) become, in this way, an active part of the conjugated system. However, with time evolution, some of the dye molecules react with the substrate (otherwise, they would be removed by washing); however, the reaction occurs slowly, after the equilibrium adsorption conformation is established and has very little or no effect on the final conformation of the dye molecule. For comparison purposes, the spectrum of a sample where pure rhodamine B physically adsorbed from an ethanolic solution with subsequent solvent evaporation is also included in Figure 7a (curve 3). It displays a slight shift toward higher BE. This is certainly due to the fact that, with this high loading of the dye, some aggregation on the external surface exists and, as a consequence, the amount of planar conformers increases relative to case 2.

Figure 7b is the same as Figure 7a for samples with lower loadings of the reactive dye (about 0.35 μmol/g of cellulose). The dyed cellulose presents the lowest BE component as before. Sample 2, where the dye was physically adsorbed from an aqueous solution and subsequently washed with water, also exhibits a shift toward higher BE as for higher loadings (see Table 3). The main difference lies on the sample prepared with pure rhodamine B from ethanolic solutions: the central peak is close to the one for sample 2, as in Figure 7b, but presents large "wings" on both sides. The large wings are indicative of very different charge densities surrounding each nitrogen. These very different charge densities are, in principle, due to a nitrogen strongly interacting with the substrate by hydrogen bonding (chemical shift toward higher binding energies)[27]—the other one dangling over the surface. This effect is hidden in samples having higher loadings of the dye,

by the large amount of molecules with a planar conformation due to aggregation. The fact that samples 1 and 2 do not show the same effect is probably due to a removal of those conformers by washing. Moreover, and since the high binding energy side is larger than the low binding energy, a higher interaction between the dye and the substrate, through hydrogen bonding, seems to occur when pure rhodamine B is adsorbed, which does not exist when isothiocyanate is used.

4. Conclusions

X-ray photoelectron spectroscopy and UV/vis absorption and luminescence studies proved to be complementary techniques in the study of a rhodamine dye adsorbed or bound to a natural polymer, microcrystalline cellulose. This allowed us to establish a clear picture of the specific interactions of rhodamine B chemically adsorbed onto the substrate.

UV/vis ground-state diffuse reflectance absorption and luminescence studies of rhodamine B showed a significant decrease of fluorescence emission quantum yield when this probe is covalently bound to microcrystalline cellulose, due to stereochemical constraints and formation of nonplanar and nonemissive conformers.

Subnanosecond fluorescence lifetime determination for the above-mentioned samples showed that rhodamine B isothiocyanate exhibits lifetimes of 3.2 ± 0.10 ns whenever this dye is physical adsorbed onto cellulose and as a monomer, while the chemically attached dyes have lifetimes of 2.5 ± 0.10 ns.

X-ray photoelectron studies provided evidence for different electronic densities around the nitrogen atom. Whenever the dye is covalently attached by the use of a dyeing method, nonplanar forms of the xanthene dye exist and the sp_3 character of the nitrogen's atoms of the amino groups increases. For physically adsorbed rhodamine B or chemically bound without the use of a dyeing methodology, molecules are able to stay in a more planar form, as shown by the decrease of the electronic density of the nitrogen atoms with an increase of sp_2 character.

All the results presented led us to conclude that dyeing reaction kinetics plays a predominant role on the formation of nonplanar conformers when the dye is covalently bound to cellulose and, in this way, deeply affects the photophysical properties of the dye.

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