CHEMPHYSCHEM

# Luminescence Quantum Yield Determination for Molecules Adsorbed onto Solid Powdered Particles

Luis F. Vieira Ferreira,* Tiago J. F. Branco, and Ana M. Botelho do Rego[a]

*A new methodology for the determination of the fluorescence quantum yield of dyes adsorbed onto microcrystalline cellulose is presented and applied to rhodamine 101, cresyl violet and auramine O. It is based on a previously reported method by Ruetten and Thomas (J. Phys. Chem., **1998**, 102, 598–606), which is not applicable to the dyes used in the present study. It uses ground-state diffuse reflectance spectra obtained with and without filters, which prevents the luminescence of the dye from reaching the integrating sphere and the photodetector.*

*New equations are presented here, correcting for the fluorescence emission of the dye, which depends on the detector sensitivity. Cut-on filters, which have a transmittance close to unity in the absorption region, and close to zero in the emission region, of the dye are used to obtain corrected reflectance spectra. The influence of the substrate was also taken into account.*

*This methodology may be applied to other probes and surfaces or emissions of a different nature (i.e., phosphorescence or delayed fluorescence), and constitutes a very simple and general procedure to solve the important problem of luminescence quantum yield determination of probes adsorbed onto solid powdered surfaces.*

## 1. Introduction

Photochemical studies of dyes and other emissive probes adsorbed onto powdered surfaces or included into cavities of these surfaces are an important field of research with growing interest in the scientific community.[1–11] These studies are important because, in many cases, adsorbed probes behave in quite a different way when compared with their behavior in solution.[7a] How does a specific surface affect an adsorbed or included probe in the ground state? How far are the photophysics and photochemistry of an excited probe modified by the interactions with a specific surface? In many cases the answers to these simple questions are far from being fully understood.

The luminescence quantum yield of probes adsorbed onto surfaces affords important information regarding the characterization of the excited state. In some cases, the increased rigidity of the adsorbed molecule results in a decrease in vibrational relaxation pathways and in a huge increase in the fluorescence quantum yield ($\Phi_F$). In the specific case of the dye auramine O adsorbed on microcrystalline cellulose, an increase of four orders of magnitude was reported relative to the dye in solution.[5b] For cyanine dyes, where isomerisation processes occur in solution, an increase of three orders of magnitude was determined for the fluorescence quantum yield of these dyes going from an ethanolic solution to adsorption on microcrystalline cellulose.[6] In the case of rigid dyes such as rhodamine 101, or moderately rigid dyes such as rhodamine 6G, no or only a very moderate change in $\Phi_F$ was found.[5a,b]

Adsorption onto powdered surfaces or inclusion into cavities of these surfaces may also be an important methodology to obtain room-temperature phosphorescence (or fluorescence) in many cases,[5d,10a,7,8] because molecules can be protected by the host from the quenching action of oxygen, which efficiently nonradiatively deactivates both triplet and singlet excited states in solution samples.

About one decade ago, we suggested a method for the determination of fluorescence of dyes adsorbed onto a powdered solid, microcrystalline cellulose, using a rigid dye, rhodamine 101, and also rhodamine 6G as fluorescence standards.[5] The main argument was that the fluorescence quantum yields were close to unity in solution, reaching in both cases, by immobilization within the cellulose polymer chains, a limiting value which was assumed to be unity.

This immobilization was achieved by the use of a good solvent in terms of the swelling of the cellulose matrix. After solvent removal, the dye molecules were rigidified into the matrix, therefore reaching a common maximum fluorescence yield. A comparison of the slopes of the intensity of fluorescence ($I_F$, measured as the area of the corrected fluorescence spectra) as a function of the light adsorbed by the dye [$(1-R)f^{dye}$, where $R$ is the total diffuse reflectance at the excitation wavelength for the probe plus substrate, and $f^{dye}$ is the fraction of the absorbed light which excites the dye] after measurements for the dye sample (unknown sample) and for the standard give us the luminescence quantum yield of the

[a] *Prof. L. F. Vieira Ferreira, T. J. F. Branco, Prof. A. M. Botelho do Rego*
*Centro de Química-Física Molecular—*
*Complexo Interdisciplinar, Instituto Superior Técnico*
*Av. Rovisco Pais, 1049-001 Lisboa (Portugal)*
*Fax: (+351) 21-846-44-55*
*E-mail: LuisFilipeVF@ist.utl.pt*

*Supporting information for this article is available on the WWW under http://www.chemphyschem.org or from the author.*

  DOI: 10.1002/cphc.200400303 

dye. $I_F$ versus $(1-R)f^{dye}$ is linear whenever the dye is present only in the monomer form. Negative deviations from linearity were found when dimer or higher aggregated forms of the dye were formed,[5,10] because these aggregates do not exhibit, in many cases, fluorescence or exhibit a very low fluorescence quantum yield of emission.

Herein, we present a new methodology for the determination of the luminescence quantum yield of dyes (or other adsorbed molecules) on surfaces. The method is based on the experimental determination of the reflectance of the adsorbed dyes as a function of concentration, with and without filters to block the luminescence emission. The luminescence emission (corrected spectra) is also needed for correcting the influence of the spectral sensitivity of the detection system.

## 2. The New Methodology

### Luminescence Quantum Yield Determination Based on Diffuse Reflectance Measurements

In an important paper, Ruetten and Thomas[3b] presented a new method to calculate fluorescence quantum yields with the use of a spectrophotometer operating with an integrating sphere. The method involves measuring the diffuse reflectance spectrum of molecules adsorbed onto powdered solid substrates in the presence and absence of a fluorescence quencher, $O_2$ in this case.

This method takes into account the fact that the integrating sphere collects not only the diffuse reflected light, but also the luminescence (fluorescence and/or phosphorescence) of the sample at the excitation wavelength. In the presence of an efficient quencher, this luminescence is quenched and an increase of absorption is observed. Therefore, Equation (1) can be used to calculate the fluorescence quantum yield $\Phi_F$ (or the luminescence quantum yield if fluorescence and phosphorescence exist for a specific sample):

$$\Phi_F = \frac{I_F}{I_{abs}} = \frac{R_u - R_{O_2}}{R_{sub} - R_{O_2}} \quad (1)$$

In this equation, the intensity of fluorescence is $I_F \propto (R_u - R_{O_2})$, and the intensity of the absorbed light is $I_{abs} \propto (R_{sub} - R_{O_2})$. $R_u$ is the reflectance of the unknown sample under study, $R_{O_2}$ the reflectance measured in the presence of the quencher, that is, when no fluorescence exists, and $R_{sub}$ is the substrate reflectance. All these reflectances should be measured at the excitation wavelength $\lambda_0$, as Figure 1 shows (in this Figure, $R_{O_2}$ is replaced by $R$).

Some problems regarding this equation should be taken into account: the equation is only valid in the case where complete quenching is reached; it also assumes that all the emitted light is detected, independently of the emission wavelength.

Equation (1) takes into account diffuse reflectance and not total reflectance (diffuse plus specular reflectances) because the specular component is eliminated by the use of a baffle, which exists inside the integrating sphere. This is certainly valid for the substrate and also for the unknown because this specular component does not exceed 1–2% of the total detected light in most cases, and is not relevant in terms of exciting the luminescent species.[12]

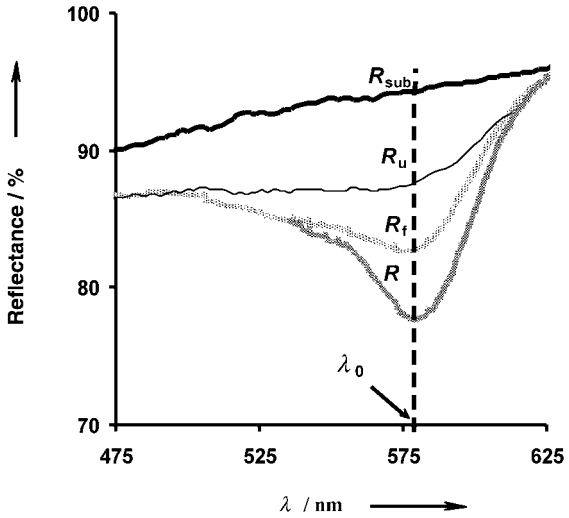


***Figure 1.*** *Ground-state diffuse reflectance spectra for rhodamine 101 adsorbed on microcrystalline cellulose, (0.5 μmol g$^{-1}$).* $R_{sub}$ *is the substrate reflectance,* $R_u$ *the unknown sample reflectance without filter,* $R_f$ *the reflectance obtained with the cut-on filter, and* R *is the corrected reflectance. All these reflectance values were measured at the excitation wavelength,* $\lambda_0$.

It is obviously difficult to insure that all the luminescent molecules are quenched by oxygen. On many surfaces oxygen does not act as a quencher: for example, in the internal channels of silicalite, a hydrophobic zeolite, molecular oxygen plays a minor role, or no role at all, depending on the loading of the fluorophore inside the nanochannels.[7] Calix[*n*]arene ($n = 4$, 6, and 8) cavities are also very good examples where $O_2$ plays no quenching role as in the case of benzophenone and benzil molecules included within these hosts.[8c]

Aromatic ketones forming inclusion complexes with cyclodextrin are also good examples of molecules that cannot be reached by molecular oxygen, therefore they may phosphoresce even in the presence of other efficient gaseous quenchers.[7b]

The second assumption in Equation (1) is certainly a very important source of errors in the calculations of $\Phi_F$, since the sensitivity of the analyzing detector varies with the wavelength,[13] as Figure 2 shows taking again rhodamine 101 fluorescence emission as an example. This fact is taken into account for the diffuse reflected light in the standard calibration procedure, made in our case with magnesium oxide, barium sulfate, or commercial standards (polymers of unknown composition supplied with an accurate calibration curve of $R$ versus wavelength in the 200–900 nm spectral range).[14]

However, when the fluorescence (or phosphorescence) emission occurs, each luminescent probe emits at a specific series of wavelengths and is detected according to the sensitivity of each specific detection system, which is wavelength dependent. Therefore, this fact has to be carefully taken into account and Equation (1) needs to be modified.

  

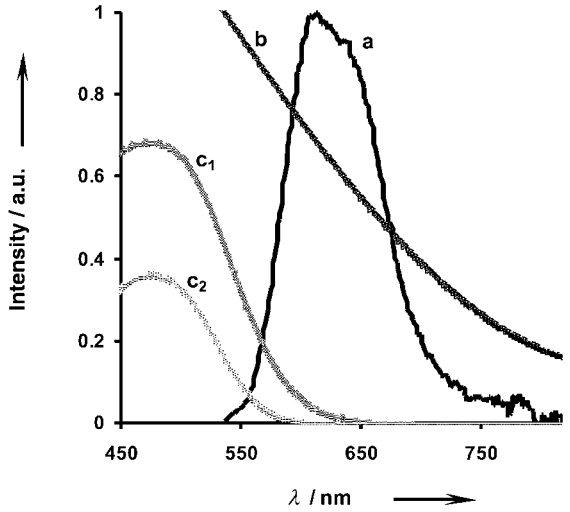


***Figure 2.*** *a) Corrected fluorescence emission spectrum of rhodamine 101 adsorbed on microcrystalline cellulose (0.5 μmol g$^{-1}$), excited at 530 nm and normalized at the maximum emission. b) Relative detector efficiency as supplied by the manufacturer, normalized to unity in the beginning of the fluorescence emission. (c1 and c2) Transmittance curves for the cut-on filters used.*

Our approach was the use of cut-on filters placed before the detector, which absorb selectively most, but not all, of the fluorescence.[5–7] The reflectance spectra are still distorted because of the residual emission reaching the photomultiplier, but this distortion is insignificant in many cases. However, for fluorophores with high quantum yields of luminescence, this approach is insufficient and the experimentally obtained reflectance has to be corrected, as we will see later in the text.

### First Correction

Taking into account the relative spectral response of the detector $S(\lambda)$, which changes with the wavelength, Equation (2) is obtained:

$$\Phi_F = \frac{I_{F_{corr1}}}{I_{abs}} = \frac{R_u(\lambda_0) - R_f(\lambda_0)}{[R_{sub}(\lambda_0) - R_f(\lambda_0)] \times f^S} \quad (2)$$

where $R_f(\lambda_0)$ is the diffuse reflectance in the presence of the cut-on filter, at the excitation wavelength $\lambda_0$.

The derivation of Equation (2) is presented in the Supporting Information. In this equation $(R_u(\lambda_0) - R_f(\lambda_0))/f^S$ is proportional to $I_F(\lambda_0)$, the intensity of the fluorescence emission (excitation at $\lambda_0$) and $(R_{sub}(\lambda_0) - R_f(\lambda_0))$ is proportional to the absorbed light at the excitation wavelength, $I_{abs}(\lambda_0)$. The factor $f^S$ is the correction introduced to take into account the variation of the spectral response of the detector, calculated with the use of Equation (3):

$$f^S = \int_\lambda I_F(\lambda) \cdot S(\lambda) d\lambda \Big/ \int_\lambda I_F(\lambda) d\lambda \quad (3)$$

Obviously $0 < f^S < 1$, the upper limit being obtained for an ideal detector, where $S(\lambda) = 1$, in all the range of detection.

### Second Correction

In Equation (2) the reflectance obtained with the cut-on filter, $R_f$, is considered to be accurate, that is, this simplified equation is valid only for situations where $R_f \approx R$, where $R$ is the reflectance obtained in the total absence of luminescence.

A more general equation should be used to obtain the luminescence quantum yields for probes adsorbed onto powdered solid substrates, in cases where $R_f \neq R$, that is, whenever the photoluminescence is significant when compared with the diffuse reflectance that reaches the detector. The more general equation is Equation (4), (where $\lambda_0$ was omitted for simplicity):

$$\Phi_F = \frac{I_{F_{corr2}}}{I_{abs}} = \frac{R_u - R_f}{(R_{sub} - R_f) \times \left[ f^S - \frac{f^{S,T}(1 - R_u)}{(1 - R_f)} \right]} \quad (4)$$

The quantity $f^{S,T}$ is defined by Equation (5):

$$f^{S,T} = \int_\lambda I_F(\lambda) \cdot S(\lambda) \cdot T(\lambda) d\lambda \Big/ \int_\lambda I_F(\lambda) d\lambda \quad (5)$$

and is a correcting factor which accounts for both the variation of the spectral sensitivity of the detector with the wavelength and the transmittance wavelength dependence, $T(\lambda)$.

The integrals of Equations (3) and (5) have to be calculated with the use of the data in Figure 2. This second correction takes into account the fact that, in most cases, the cut-on filters are not "ideal" cut-on filters, which completely remove the fluorescence from the integrating sphere, and thus in practice some fluorescence still reaches the detector.

The derivation of Equation (4), including the first and second corrections for $\Phi_F$, is presented in the Supporting Information. Another very important relation is Equation (6):

$$(R_{sub} - R_f) = f^{dye} \times (1 - R_f) \quad (6)$$

where $f^{dye}$ is the fraction of the absorbed photons which excite the dye (or probe in general), and not the substrate, at the excitation wavelength, $\lambda_o$, defined by Equation (7):

$$f^{dye} = \frac{F(R,\lambda_0)_{dye}}{F(R,\lambda_0)_{total}} \quad (7)$$

and $f^{sub} = (1 - f^{dye})$ is the homologous quantity for the substrate. $F(R,\lambda_0)$ is the remission function defined as $(1-R)^2/2R$.[2,12]

Equations (2) and (4) take into account the fact that for each luminescent sample the photodetector "sees" the diffuse reflected light plus some luminescence which depends on the absorbed light $I_{abs} = I_0(1 - R(\lambda_0))$, on the fraction of the absorbed photons which excite the dye $f^{dye}$, and also on the quantum yield of emission $\Phi_F$. Therefore $R_u$ is $R$ plus the luminescence contribution. This second part is affected by the sensitivity of the detector, so this effect has to be corrected by $f^S$. For a non-luminescent sample, $R_u = R$. The same applies to the reflectance measured with a good cut-on filter. For an "ideal" cut-on filter, $R_f = R$.

  

In the Supporting Information, we also have shown that both $f^{dye}$ and the quantity $R$ can be calculated allowing for the correction of the diffuse reflectance spectra. However, these corrected values are not needed for the $\Phi_F$ calculation, as Equations (2) and (4) clearly show.

## 3. Results and Discussion

### Rhodamine 101

Diffuse reflectance spectra obtained for rhodamine 101 adsorbed on microcrystalline cellulose, with a cut-on filter (thick curves) and without filter (thin curves) are shown in Figure 3.

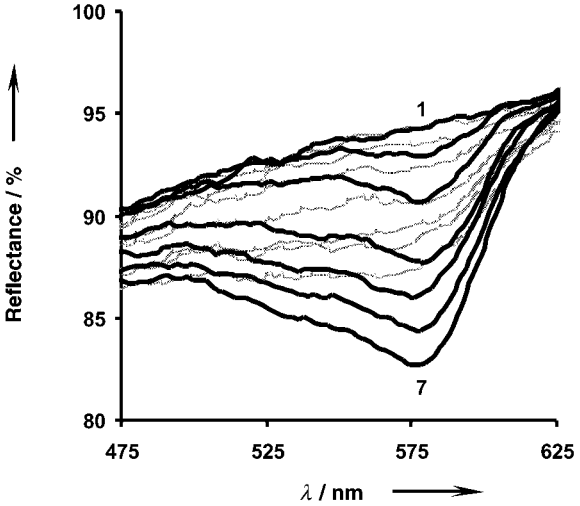


*Figure 3. Diffuse reflectance spectra obtained for rhodamine 101 adsorbed on microcrystalline cellulose, with the c1 cut-on filter (thick curves) and without filter (thin curves). Curves 1–7 are for 0, 0.005, 0.01, 0.05, 0.10, 0.25, and 0.50 μmol dye per gram of cellulose, respectively.*

The dye loading increases from curve 1 (microcrystalline cellulose with no dye) to curve 7, the highest concentration of the dye (0.50 $\mu mol\,g^{-1}$). A significant decrease in the reflectance values was obtained with the use of the cut-on filter, $R_f$, relative to the one obtained without the use of the filter, $R_u$, showing the distortion due to the fluorescence of rhodamine 101. These $R_f$, $R_u$ and $R_{sub}$ can be used in Equations (2) and (4), together with the correcting factors for the variation of the spectral sensitivity of the detector, $f^S$, and for the filter transmittance $f^{S,T}$, to calculate the fluorescence quantum yield of rhodamine 101 adsorbed onto the powdered solid substrate, as Figure 4 shows in curves B and C, respectively.

Figure 4a presents data with the use of the cut-on filter c1 of Figure 2, and Figure 4b shows the equivalent data obtained with a more "severe" cut-on filter, curve c2 of Figure 2. In both Figures 4a and 4b, the intensity of fluorescence $I_F$—measured as $(R_u-R_f)$, uncorrected values, $(R_u-R_f)/f^S$, values with the first correction, and $(R_u-R_f)/[f^S-f^{S,T}(1-R_u)/(1-R_f)]$ values with the second correction at the excitation wavelength $\lambda_o$—is plotted as a function of the fraction of the excitation light which is absorbed by the dye in the sample (measured as $R_{sub}-R_f=(1-R_f)f^{dye}$). The relation between these two quantities was established in Equation (8) for probes adsorbed onto powdered solid surfaces:[12,5]

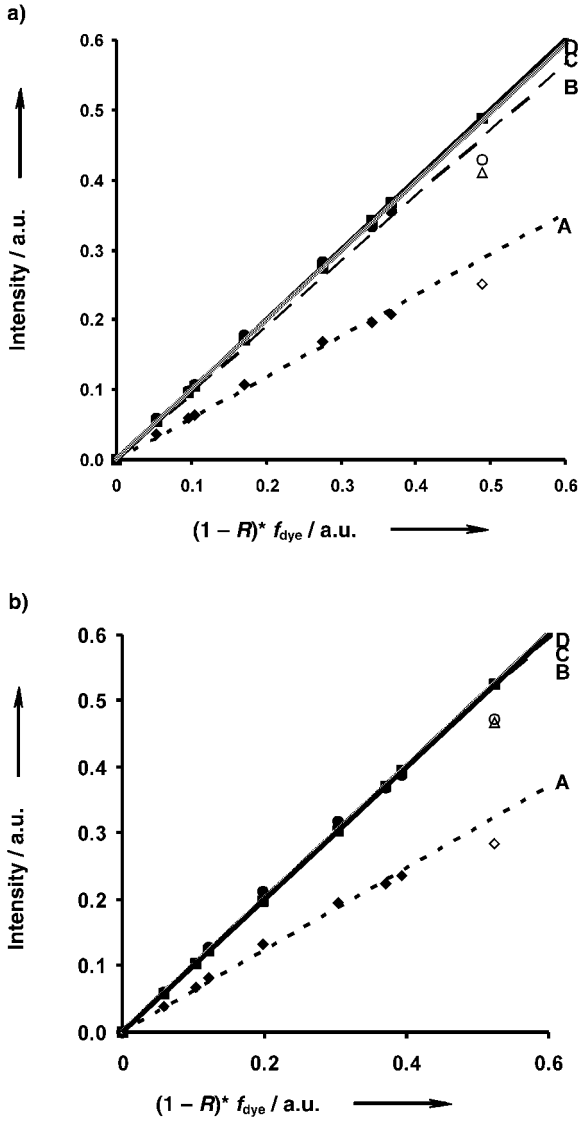


*Figure 4. Intensity of fluorescence ($I_F$) of rhodamine 101 adsorbed onto microcrystalline cellulose, excited at 530 nm, as a function of the light absorbed by the dye ($I_{abs}$) which is proportional to $[1-R(\lambda_0)]f^{dye}$, $\lambda_0=580$ nm. Curve A is for $I_F \propto [R_u(\lambda_0)-R_f(\lambda_0)]$, and curve D is for $I_{abs} \propto [R_{sub}(\lambda_0)-R_f(\lambda_0)]$. Curve B was obtained by introducing the first correction [Equation (2)] and curve C with the second correction [Equation (4)]. The corrected fluorescence quantum yield is obtained by the ratio of the slopes of curves C and D. Full symbols were used for the regression and empty symbols were not taken into account. Part a) of Figure 4 was obtained by the use of transmission filter c1, and part b) by the use of filter c2 of Figure 2.*

$$I_F = G\Phi_F I_0(1-R_f)f^{dye} \quad (8)$$

where $G$ is a geometrical factor that depends on the apparatus and the other parameters were as defined previously. Plots of $I_F$ versus $(1-R_f)f^{dye}$ show linear increases for low loadings of the dye, and deviations from linearity were observed for the higher loadings. Such deviations may be due to dye aggrega-

  

tion that usually decreases the fluorescence emission,[5] or to fluorescence reabsorption.[15]

In Figure 4, $I_F$ versus $(1-R_f)f^{dye}$ is represented by curve A. Its slope should give the fluorescence quantum yield if no correction was needed. Data including the first correction for the reflectance are represented by curve B. The use of the second correction [Equation (4)] transforms curve B into curve C, and obviously this second correction is more important where the cut-on filter did not eliminate the fluorescence of the dye so efficiently, that is, the case of filter c1. In cases where the dye has a unitary quantum yield of fluorescence, curves C and D of Figure 4 should be coincident and have a slope with a unitary value and that is indeed the case for rhodamine 101 adsorbed onto microcrystalline cellulose.

The important point to emphasize from these data, is that a common value for $\Phi_F$ was obtained with the use of the two different filters: $0.994 \pm 0.012$ or $1.009 \pm 0.013$, showing that the proposed methodology for the determination of $\Phi_F$ is valid and also in accordance with the previous reported approach for the determination of $\Phi_F$ of rhodamine 101 adsorbed onto microcrystalline cellulose.[5a]

### Cresyl Violet and Auramine O

This approach was also applied to two other dyes: cresyl violet and auramine O. The former dye is commonly used as a standard for solution studies of fluorescence quantum yield determination:[16] its fluorescence quantum yield being slightly dependent on the wavelength used for excitation (0.51 in ethanol for excitation at 546 nm).[16] Auramine O is well known for its high mobility in solution, the nonradiative singlet decay route depending on the internal rotation of the phenyl groups and is very sensitive to the environment, reducing its fluorescence quantum yield to less than 0.001 in solvents such as water, ethanol or butanol and increasing slightly in glycerol.[17, 5b]

Similar data to those presented in Figure 4 for rhodamine 101 were obtained for these two dyes. Emission spectra of auramine O and cresyl violet can be found, for instance, in refs. [5b], [16b].

A final correction is also needed to account for the reabsorption of the dye fluorescence by the ground-state dye (self-absorption) especially when the fluorescence quantum yield is far from 100%. This can be done according to the work of Oelkrug and Kortum, who developed an equation for self-absorption correction in powdered samples,[12] (Equation A5 in the Supporting Information) where $R$ is the reflectance at the excitation wavelength $\lambda_0$ and $R_{anal}$ is the reflectance at the analyzing wavelength.

Equations A6 and A7 were later obtained by a different approach and published with a different formal presentation by other authors (Lagorio and San Roman)[15] and can be used to correct $\Phi_F$ for self-absorption in solid powdered samples, calculating $I_F^{corr}$ from $I_F^{obs}$. Here we do not have a set of emission spectra, and the purpose of this work is the presentation of a new method for the calculation of $\Phi_F$ from ground state diffuse reflectance studies. Therefore a simpler equation can be used, that is, Birk's equation (see Equation A8 in Supporting Information) where $\alpha$ is the probability of self-absorption of an emitted photon (it is assumed here that the probability for first reabsorption is equal to the second and so on). This simple equation was used herein, in a previous paper by us[10a] and by other authors,[15] and the results of these calculations are presented in Table 1. The probability $\alpha$ was evaluated by comparing the area of the corrected emission spectra for a very diluted solid powdered sample with another where reabsorption occurs, normalizing the two spectra at longer wavelengths, where the spectrum is not affected by reabsorption. $\Phi_F^{obs}$ will be smaller than $\Phi_F^{corr}$ in all cases, except in the case where $\Phi_F^{corr} = 1$, which is indeed the case of rhodamine 101 adsorbed onto microcrystalline cellulose.

***Table 1.*** *Fluorescence quantum yields for the three dyes under study, with and without corrections (self-abs. = self-absorption; t = t-student's parameter).*

| Dye[a] | Eq. (1) | Eq. (2) 1st correction | Eq. (4) 2nd correction | Eq. A5 with self-abs. correction | Literature | Standard Deviation | *t* (95%) |
|---|---|---|---|---|---|---|---|
| R 101 (C1) | 0.586 | 0.941 | 0.994 | 0.994 | 1.00 | 0.012 | 2.5 |
| R 101 (C2) | 0.620 | 0.995 | 1.009 | 1.009 | 1.00 | 0.013 | 2.5 |
| CV | 0.235 | 0.393 | 0.408 | 0.504 | 0.51 | 0.011 | 2.6 |
| AO | 0.302 | 0.408 | 0.432 | 0.472 | 0.35[b] | 0.046 | 4.4 |

[a] Dyes: rhodamine 101 (R 101); cresyl violet (CV); and auramine O (AO). [b] From ref. [5b], that is, without corrections for the detector sensitivity and filter transmittance.

Table 1 summarizes the fully corrected fluorescence quantum yields determined for the three dyes under study and compares the data obtained herein with and without the self-absorption correction and also with literature data. A detailed statistical treatment of the data was done to validate quantitatively our proposed methodology. For rhodamine 101 the source data is Figure 2 at 580 nm, while for all other entries similar reflectance curves were used.

For cresyl violet, the use of the first and second corrections increases $\Phi_F$ from 0.235 to 0.393 and 0.408, respectively. Following further introduction of the self-absorption correction [Equation A5], $\Phi_F$ reaches $0.504 \pm 0.011$, which is comparable to the literature value for this dye obtained for solution studies.[16] However, the $\Phi_F$ value obtained herein refers to a different excitation wavelength (540 nm) whereas the one included in Table 1 from literature was obtained with 546-nm excitation in ethanol and a $\Phi_F$ dependence on the excitation wavelength was well documented before.[16] $\Phi_F$ values in solution and on the solid are similar, as in the case of rhodamines 101 and 6G. This is probably due to the structural rigidity of these molecules, and entrapment into the polymer chains of microcrystalline cellulose did not affect the nonradiative pathways of deactivation significantly.

  

For auramine O the obtained $\Phi_F$ uncorrected value, $0.302 \pm 0.029$, agrees quite well with the value previously found by us using a different method.[5b] In that paper[5b] we did not take into account the correction for the detector sensitivity and for the filter transmittance presented here. We previously obtained a value of 0.35 after very prolonged drying in the vacuum chamber and we now obtain $0.302 \pm 0.029$; both values are before the application of the first and second corrections. The application of these corrections increases the $\Phi_F$ to 0.408 and 0.432, respectively. After the self-absorption correction of the later, $\Phi_F$ reaches the value of $0.472 \pm 0.046$.

## 4. Conclusions

A new methodology was established for fluorescence quantum yield determination of dyes adsorbed onto solid powdered substrates. It can be used for any other compound adsorbed onto scattering adsorbents, provided the adsorbed probe stays in the monomer form in the concentrations under study. However, aggregation effects can be taken into account in the calculations by considering fractions of the light adsorbed by monomers, dimers or higher order aggregates. It can also be applied to the phosphorescence quantum yield determinations, as in the case of room temperature phosphorescent samples.

It uses ground-state diffuse reflectance spectra obtained with, and without, cut-on filters, which prevent the luminescence of the dye to reach the integrating sphere and the photodetector. New equations were presented here, correcting for the fluorescence emission of the dye, which affects the measured reflectance and depends on the detector sensitivity. There is no need to use luminescence standards with this new methodology, which can be used for all luminescent systems, including those where oxygen does not completely quench the photoluminescence.

The fluorescence quantum yields of rhodamine 101, cresyl violet and auramine O adsorbed onto microcrystalline cellulose were determined ($1.009 \pm 0.013$, $0.504 \pm 0.011$, $0.472 \pm 0.046$, respectively) and were in agreement with previous reported data obtained by other methodologies.

## Experimental Section

Materials: Rhodamine 101 perchlorate (Radiant Dyes Chemie) and cresyl violet perchlorate (Aldrich) were both laser dye grade and used as supplied. Auramine O (Aldrich) was purchased as a certified dye and purified according to ref. [5b]. Ethanol, (Merck, Uvasol grade) was used as received. Microcrystalline cellulose (Fluka DS-O) with 50 μm, average particle size was used as the solid substrate also without further purification.

Sample Preparation: The samples used herein were prepared using the solvent evaporation method. This method consists of the addition of a solution containing the probe to the previously dried powdered solid substrate, followed by solvent evaporation from the stirred slurry in a fume cupboard. Ethanol was used for sample preparation because it swells cellulose, that is, the hydrogen bonds between the hydroxyl groups of the polymer chains are replaced by a chain–solvent–chain interaction,[5d] allowing the guest molecules to diffuse into the matrix. These guest molecules stay entrapped after solvent removal.[5d] The final solvent removal was performed overnight in an acrylic chamber with an electrically heated shelf (Heto, Model FD 1.0–110) with temperature control ($30 \pm 1$ °C) and under moderate vacuum at a pressure of approximately $10^{-3}$ Torr. The evaluation of the existence of final traces of solvent was monitored by the use of FTIR spectroscopy.

Ground-State Diffuse Reflectance Absorption Spectra (GSDR): Ground-state absorption spectra for the solid samples were recorded using an OLIS 14 spectrophotometer (based on a Cary 14) with a diffuse reflectance attachment. Further details are given elsewhere.[5a,b,d] The diffuse reflectance attachment used in the Cary 14 is described in detail in ref. [12b]. The integrating sphere includes a baffle and can be used in two different positions, allowing the detection of the specular light or avoiding it. In this way, we obtained 1–2% of specular radiation only.
The cut-on filters used in this work were all supplied by Corion. The short-pass filter (reference 575GK50) and the band-pass filter (reference 475GB50) were denoted as c1 and c2, respectively. For the case of auramine O a short-pass filter was also used (reference 4655GK50)

Steady-State and Laser-Induced Luminescence (LIL) Systems: Corrected fluorescence emission spectra (steady state) were obtained with a homemade apparatus described elsewhere.[5c] Schematic diagrams of the LIL system are presented in refs. [1], [7b]. The light arising from the irradiation of solid samples by the laser pulse is collected by a collimating beam probe coupled to an optical fiber (fused silica) and is detected by a gated intensified-charge-coupled device, Oriel model Instaspec V, (Andor ICCD, based on the Hamamatsu S57 69-0907). The ICCD is coupled to a fixed-imaging compact spectrograph (Oriel, model FICS 77441). The system was used for laser-induced luminescence experiments with a $N_2$ laser (PTI model 2000, ca. 600 ps FWHM, $\approx$1.1 mJ per pulse), as the excitation source. In this case the excitation wavelength is 337 nm. With these setups, both fluorescence and phosphorescence spectra are easily available (by the use of the variable time gate width and start delay facilities of the ICCD).

## *Acknowledgements*

*T.J.F.B. thanks the FCT for a Doctoral fellowship SFRH/BD/8143/2002.*

**Keywords:** absorption · diffuse reflectance spectra · fluorescence · luminescence · microcrystalline cellulose

[1] a) L. F. V. Ferreira, *Química* **1999**, *72*, 28–46; b) A. M. B. Do Rego, L. F. V. Ferreira in *Handbook of Surfaces and Interfaces of Materials, Vol.* 2 (Ed.: H. S. Nalwa), Academic Press, San Diego, **2001** , pp. 275–313.
[2] a) F. Wilkinson, G. P. Kelly in *Photochemistry on Solid Surfaces* (Eds.: M. Anpo, T. Matsuara), Elsevier, Amsterdam, **1989**, pp. 31–47; b) F. Wilkinson, G. P. Kelly in *Handbook of Organic Photochemistry, Vol.* 1 (Ed.: J. C. Scaiano), CRC Press, Boca Raton, **1989**, pp. 293–314.
[3] a) J. K. Thomas, *Chem. Rev.* **1993**, *93*, 301–320; b) S. A. Ruetten, J. K. Thomas, *J. Phys. Chem. B* **1998**, *102*, 598–606; c) Y. Mao, J. K. Thomas, *J. Chem. Soc. Faraday Trans.* **1992**, *88*, 3079–3086; d) Y. Mao, G. Zang, J. K. Thomas, *Langmuir* **1993**, *9*, 1299–1305; e) Y. Mao, J. K. Thomas, *Langmuir*, **1992**, *8*, 2501–2508.
[4] J. H. Hurtubise, *Anal. Chim. Acta* **1997**, *351*, 1–22.
[5] a) L. F. V. Ferreira, M. R. Freixo, A. R. Garcia, F. Wilkinson, *J. Chem. Soc. Faraday Trans.* **1992**, *88*, 15–22; b) L. F. V. Ferreira, A. R. Garcia, M. R. Freixo, S. M. B. Costa, *J. Chem. Soc. Faraday Trans.* **1993**, *89*, 1937–1944;

  

c) L. F. V. Ferreira, S. M. B. Costa, E. J. Pereira, *J. Photochem. Photobiol. A* **1991**, *55*, 361 – 376; d) L. F. V. Ferreira, J. C. Netto-Ferreira, I. Khmelinskii, A. R. Garcia, S. M. B. Costa, *Langmuir* **1995**, *11*, 231 – 236.

[6] a) L. F. V. Ferreira, A. S. Oliveira, F. Wilkinson, D. R. Worrall, *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 1217 – 1225; b) A. S. Oliveira, L. F. V. Ferreira, D. R. Worrall, F. Wilkinson, *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 489 – 4814.

[7] a) L. F. V. Ferreira, M. R. V. Ferreira, A. S. Oliveira, J. C. Moreira, *J. Photochem. Photobiol. A* **2002**, *153*, 11 – 18; b) L. F. V. Ferreira, A. S. Oliveira, J. C. Netto-Ferreira in *Fluorescence Microscopy and Fluorescent Probes 3* (Ed.: A. Kotyc), Espero Publishing, Prague, **1999**, pp. 199 – 208.

[8] a) L. F. V. Ferreira, M. R. V. Ferreira, J. P. Da Silva, I. F. Machado, A. S. Oliveira, J. V. Prata, *Photochem. Photobiol. Sci.* **2003**, *2*, 1002 – 1010; b) L. F. V. Ferreira, M. R. V. Ferreira, A. S. Oliveira, T. J. F. Branco, J. V. Prata, J. C. Moreira, *Phys. Chem. Chem. Phys.* **2002**, *4*, 204 – 210; c) L. F. V. Ferreira, I. F. Machado, A. S. Oliveira, M. R. V. Ferreira, J. P. Da Silva, J. C. Moreira, *J. Phys. Chem.* B, **2002**, *106*, 12584 – 12593.

[9] B. Q. Ma, L. F. V. Ferreira, P. Coppens, *Org. Lett.* **2004**, *6*, 1087 – 1090.

[10] a) F. Wilkinson, P. A. Leicester, L. F. V. Ferreira, V. M. R. Freire, *Photochem. Photobiol.* **1991**, *54*, 599 – 608; b) F. Wilkinson, G. P. Kelly, L. F. V. Ferreira, V. Freire, M. I. Ferreira, *J. Chem. Soc. Faraday Trans.* **1991**, *87*, 547 – 552.

[11] a) J. P. Da Silva, A. M. Da Silva, I. V. Khmelinskii, J. M. G. Martinho, L. F. V. Ferreira, *J. Photochem. Photobiol. A* **2001**, *142*, 31 – 37; b) J. P. Da Silva, L. F. V. Ferreira, A. M. Da Silva, A. S. Oliveira, *J. Photochem. Photobiol. A* **2002**, *151*, 157 – 164.

[12] a) D. Oelkrug, G. Kortum, *Z. Phys. Chem.* **1968**, *58*, 181 – 188; b) W. W. Wendlandt, H. G. Hecht in *Reflectance Spectroscopy,* Interscience Publishers, New York, **1969**, pp. 114 – 118.

[13] R. Gade, U. Kaden, *J. Chem. Soc. Faraday Trans.* **1990**, *86*, 3707 – 3712.

[14] *Reflectance Calibration Standards*, Labsphere, Inc. (Labsphere product manual).

[15] a) M. G. Lagorio, L. E. Dicelio, M. I. Litter, H. San Roman, *J. Chem. Soc. Faraday Trans.* **1998**, *94*, 419 – 425; b) S. Amore, M. G. Lagorio, L. E. Dicelio, H. San Roman, *Prog. React. Kinet. Mech.* **2001**, *26*, 159 – 177; c) M. G. Lagorio, H. San Roman, *J. Chem. Educ.* **2002**, *79*, 1362 – 1367.

[16] a) J. Olmsted, *J. Phys. Chem.* **1979**, *83*, 2581 – 2584; b) D. Madge, J. H. Brannon, T. L. Cremers, J. Olmsted, *J. Phys. Chem.* **1979**, *83*, 696 – 699; c) S. J. Isak, E. M. Eyring, *J. Phys. Chem.* **1992**, *96*, 1738 – 1742; d) D. F. Eaton in *Handbook of Organic Photochemistry, Vol.* 1 (Ed.: J. C. Scaiano), CRC Press, Boca Raton, **1989**, pp. 231 – 239.

[17] a) G. Oster, Y. Nishizima, *J. Am. Chem. Soc.* **1956**, *78*, 1581 – 1584; b) R. F. Chen, *Arch. Biochem. Biophys.* **1977**, *179*, 672 – 681.

Received: June 28, 2004
Revised: September 21, 2004

Early View Article
Published online on November 18, 2004

  