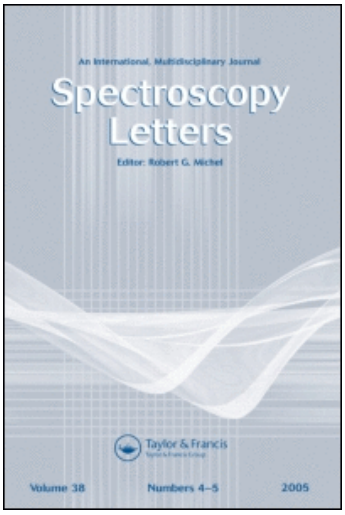


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# Aqueous Photophysical Parameters of Congo Red

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# Aqueous Photophysical Parameters of Congo Red

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**ABSTRACT** Using steady-state fluorescence spectroscopy and UV-Vis absorbance spectrometry, the salient photophysical parameters of Congo red, a very important biological staining reagent, was determined. Its absorbance was observed at 497.0 nm; its molar absorptivity $\varepsilon$ was determined as $6.26 \times 10^4$ M–cm. Its fluorescence, when excited at 330.0 nm, was observed at 417.0 nm. The quantum yield, $\phi$, in aqueous solution was determined by two different methods—the relative, or comparative, method and the absolute method. Both methods gave the same value of 0.011. The room temperature fluorescence lifetime $\tau_o$ was determined as 2.8 ns using the Strickler–Berg equation.

**KEYWORDS** absorptiometry, florescence, lifetime, molar absorptivity, photophysical parameters, quantum yield



## INTRODUCTION

Congo red, whose chemical structure is shown in Fig. 1, is a symmetric, sulfonated azo dye and belongs to the class of protein-binding dyes. The color of the solution of Congo red is pH dependent. At pH up to 3, this solution is bluish, at pH above 5, the color becomes bright red. As a result, it has been used in chemistry as a pH indicator.[1,2] Congo red is also used as a dyeing agent in the textile industry.[3–5] Perhaps the greatest use of Congo red is in biology and histochemistry. Under a polarized light Congo red is birefringent.[6,7] Congo red is known to bind to amyloid proteins,[8–12] and its birefringence under a polarized light is a characteristic feature that has made it useful in characterizing its interaction with amyloid formation in Alzheimer patients.[13] The interaction, formation, binding, detection, and aggregation of amyloid proteins has been carefully studied by spectroscopy and computer analysis[14–25,27,28] with the aid of Congo red. Congo red is known to bind specifically to the $\beta$-conformation[29,30] of amyloids, even though there is mounting disagreement of this fact. The electrostatic binding of Congo red to other forms of protein, such as the cytoskeletal, prion, insultin, etc, and the study of Congo red interaction with the $\alpha$-form of these proteins have been carried out to demonstrate its nonspecific binding to the $\beta$-amyloid fibrils.[31,32] In addition to the binding of amyloid proteins,[17,33] this compound has also been used in medicine and in chemical catalysis.[34,35] Although the biological use of Congo red as a staining agent for amyloid proteins and its modifications

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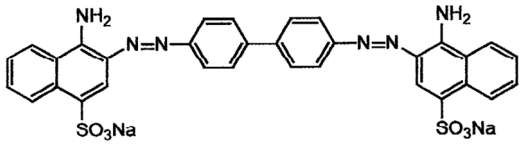

Congo Red (CR)

**FIGURE 1** The structure of Congo red.

have been intensely studied[36–45] the photophysical properties of this compound, to the author's knowledge, have not been systematically determined or recorded. Therefore, it is the theme of this study to determine these properties as a contribution to the mounting knowledge of this compound in its use not only in biology and histochemistry but also in chemistry, medicine, and biotechnology, in general.

## MATERIALS AND METHODS

The experimental materials of Congo red, concentrated $H_2SO_4$, and quinine sulfate were of reagent grade and were obtained from Fisher Scientific, Pittsburgh, PA, USA, and used without further purification.

### Optical Measurements

All fluorescence measurements were performed using a Perkin Elmer Luminescence Spectrophotometer, Model LS 50B (Waltham, MA, USA). The excitation of Congo red was carried out at 330 nm. This wavelength was used throughout the experiment because the maximum emission was observed when this wavelength was used in accordance with what the instrument put out. The excitation and emission slits were kept constant at 5.0 nm. At this excitation wavelength, the fluorescence of this compound was observed at 417 nm. In all experiments, unless otherwise specified, the concentration of Congo red was kept constant at $1.78 \times 10^{-6}$ M. All experiments were conducted at room temperature ($25.0 \pm 0.2$°C). The absorptiometric experiments were performed using a Cary Spectrophotometer, model 1E, supplied by Varian Analytical Instruments (Varian Walnut Creek, California, USA). The absorbance spectrum of this compound, whose $\lambda_{max}$ is observed at 497.0 nm, was also obtained using a 1.0-cm cuvette.

### Molar Absorptivity

The molar absorptivity $\varepsilon$ of Congo red was determined by plotting the ratio of the observed absorbance A, taken at $\lambda_{max}$ to the concentration C, as a function of the respective wavelengths encompassing the absorbance spectrum in accordance with BeerLambert's law ($\varepsilon = A/bC$). The solution thickness or light path length b was 1.0 cm. The $\varepsilon$ was taken at the peak of the plot at which $\lambda = \lambda_{max}$. This is shown in Fig. 2.

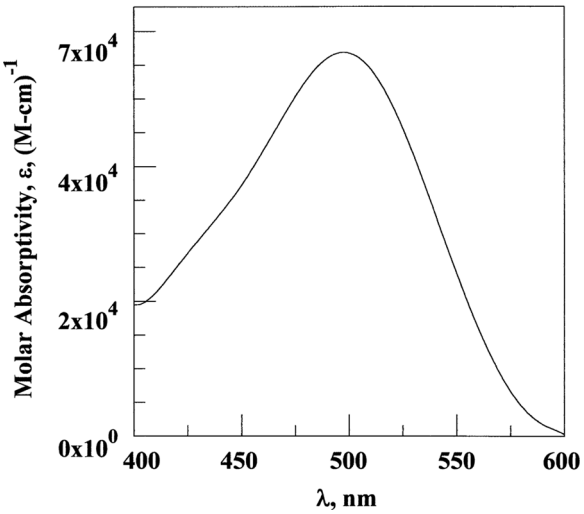


**FIGURE 2** Molar absorptivity of Congo red as a function of wavelength $\varepsilon = 6.26 \times 10^4$/M-cm.

### Refractive Index Determination

The refractive index n of plain water and that of 1.0 M $H_2SO_4$ solutions were determined using the digital Abbe Leica Refractometer, which was calibrated with a triply distilled deionized water. The obtained refractive indices for these solvents were 1.33261 and 1.34323, respectively. These are in reasonable agreement with the literature values of 1.33239 and 1.3323[46,47] for water and 1.34369 for 5.33% of $H_2SO_4$ calculated from the data of Palmer and Williams.[48]

## RESULTS

### Absorbance Spectrum of Congo Red

Congo red exhibits an uncomplicated single absorption band whose maximum centered at 497 nm, as can be seen in Fig. 3. Subsequent calculations will be based on this value, which is taken as its $\lambda_{max}$.

### Quantum Yield

The quantum yield $\Phi$ of Congo red was determined by two different methods in accordance with

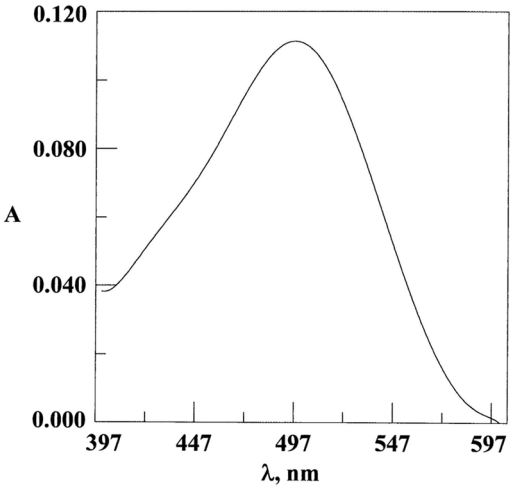


**FIGURE 3** Absorbance versus wavelength for $1.78 \times 10^{-6}$ M Congo red.

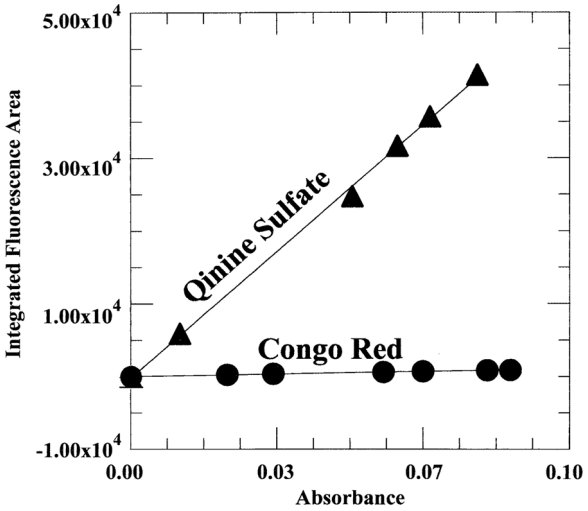


**FIGURE 4** The integrated fluorescence data versus the absorbance data for the sample and reference (see text).

the literature methodology.[49,50] Briefly, in the first method, the solutions of the reference standard (quinine sulfate) and the Congo red were prepared to have approximately equal absorbancy, 0.08, and both were excited at the same wavelength (330.0 nm). In a review article on quantum yields determination, Demas and Crosby[51] comment that the quantum yield of quinine sulfate is constant between the excitation wavelength of 200 nm and 390 nm. The value of the quantum yield for this reference sample is recommended to be 0.54, and this value was used in this work. The observed fluorescence was corrected with respect to the solvents' refractive indices n (reference and sample solutions) as shown in Eq. (1):[52]

$$\Phi_u = \Phi_s \frac{(1 - 10^{-A})F_u n_u^2}{(1 - 10^{-A})F_s n_s^2} \qquad (1)$$

In this equation, the subscripts "u" and "s" refer to the unknown sample (Congo red) and reference sample (quinine sulfate), respectively. The letters A and F refer to the absorbance and area of the integrated fluorescence band, respectively. The values of $A_s$, $A_u$, $F_s$, and $F_u$ are 0.0633, 0.0666, 35,694.60, and 735.92, respectively, and the values of the n ratio of the refractive indices of the solvents are as given above. The ratio $\frac{n_u^2}{n_s^2}$ is 0.9887.

Using the above equation, the quantum yield for Congo red was determined as 0.011. In the second method, the ratio of the gradients (868.04 and 41,449.20, respectively) of the integrated fluorescence spectrum versus the absorbance of the reference and that of the unknown solution were obtained as per Eq. (2) (Fig. 4):

$$\phi_u = \phi_s \frac{\text{gradient}_s\, n_u^2}{\text{gradient}_u\, n_s^2}. \qquad (2)$$

Again, the calculated quantum yield is 0.011.

## Fluorescence Lifetime

The fluorescence lifetime $\tau_o$ for Congo red was determined using the Strickler–Berg relation[53] given in Eq. (3):

$$1/\tau_o = 2.88 \times 10^{-9} n^2 \bar{\nu}_f^3 \frac{g_l}{g_u} \int \varepsilon d\ln\nu. \qquad (3)$$

In this equation n, $\overline{\nu}_f$, and $\upsilon$ are the solvent refractive index, the fluorescence wave number taken at the center of gravity of the fluorescence spectrum, and the integrated wave number of the absorption band, respectively. The factors $g_u$ and $g_l$ are the degeneracies of the upper and lower electronic bands, respectively. The integration in Eq. (3) was done analytically by measuring the area enclosed under the absorbance envelope in Fig. 3 using the relation of full width at half maximum (FWHM) technique. In this case, Eq. (3) is reduced to a much simpler relation, given in Eq. (4):

$$1/\tau_o = 2.88 \times 10^{-9} n^2 \bar{\nu}_f^3 \varepsilon d\ln \frac{\nu_1}{\nu_2} \qquad (4)$$

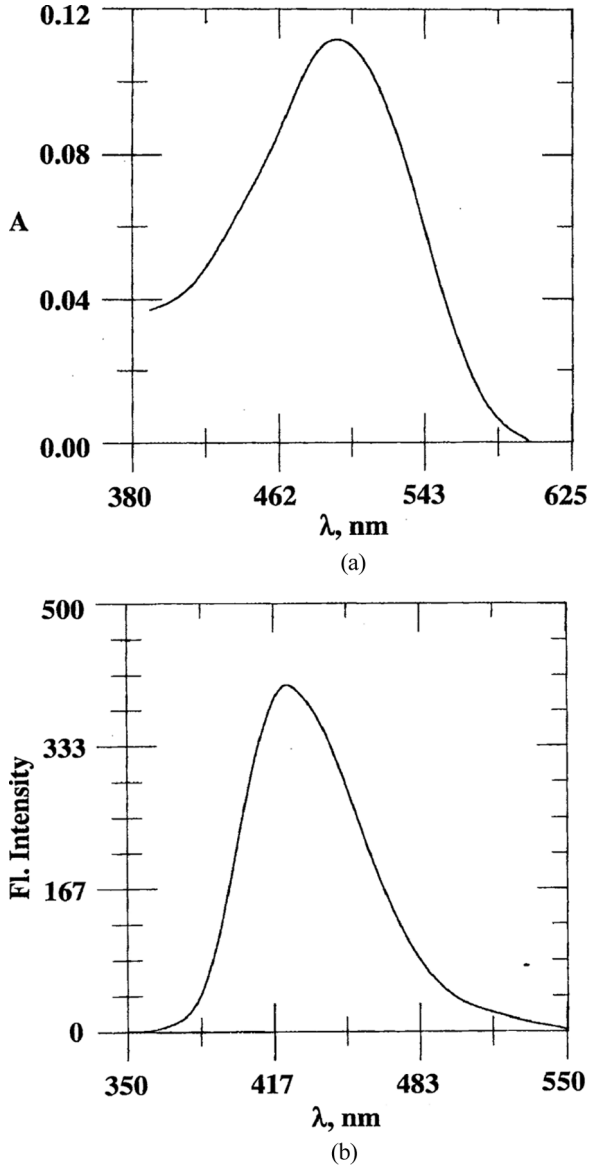


**FIGURE 5** The absorbance (a) and the florescence (b) of Congo red.

Subscripts 1 and 2 in this equation denote the wavenumber corresponding to the high and low end of the wavenumbers of the band, respectively. The shape of the ground state and the excited spectra of Congo red are almost alike (see Fig. 5). For this reason, it is assumed that the $g_l/g_u$ is unity, and therefore, $\phi^{-1}$ is not taken into consideration in the calculation of the fluorescence lifetime of Congo red.

## DISCUSSION

The absorbance and the fluorescence spectra of Congo red are shown in Fig. 5. As can be seen, the shape of the ground and excited states of this compound are almost alike. However, Fig. 3 shows the absorbance spectrum that was plotted as described in the experimental section. From this spectrum, the molar absorptivity of this compound is determined to be $6.26 \times 10^4$ M–cm at $\lambda_{max}$, which is 497 nm. This important parameter of Congo red is listed in Table 1. Using this value together with the value of the refractive index of the solvent (water), the room temperature fluorescence lifetime of Congo red was determined with the aid of the Strickler–Berg relation. The value determined is 2.8 ns which is in good agreement with that determined in ethanol solution (2.3 ns).[54] This value, 2.8 ns, is also listed in Table 1. The quantum yield of Congo red was also determined as per the methods described in the materials and methods section. The fact that the two methods that are used in this determination gave the same value (0.011) gave credence to this value, which is also listed in Table 1.

**TABLE 1** Determined Photophysical Parameters of Congo Red

| Parameter | Symbol | Value |
|---|---|---|
| Absorbance | A | 497 nm |
| Molar absorptivity | $\varepsilon$ | $6.46 \times 10^4\,M^{-1}\,cm^{-1}$ |
| Quantum yield | $\phi$ | 0.011 |
| Aqueous fluorescence lifetime | $\tau_o$ | 2.8 ns |

## CONCLUSION

The salient photophysical parameters, such as the molar absorptivity $\varepsilon$, quantum yield $\phi$, and fluorescence lifetime $\tau_o$, of Congo red were determined using steady-state fluorescence and UV-Vis absorption spectroscopy. The values obtained from these spectroscopic measurements are $6.26 \times 10$ M cm, 0.011 ns, and 2.8 ns, respectively. The obtained lifetime fluorescence value is quite comparable to a literature value.

## ACKNOWLEDGMENT

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