Journal of Inclusion Phenomena and Macrocyclic Chemistry **40:** 213–219, 2001.

The Effect of Cyclodextrins on the Photochemical Stability of 7-Amino-4-methylcoumarin in Aqueous Solution

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(Received: 19 December 2000; in final form: 11 May 2001)

Key words: photochemistry, laser dye, coumarin 120, cyclodextrins, inclusion compounds

Abstract

The effect of α-, β- and γ-cyclodextrin on the photochemical stability of 7-amino-4-methylcoumarin (C120) was studied. Using spectroscopic techniques (UV/Vis absorption spectroscopy, fluorescence, fluorescence anisotropy and circular dichroism) combined with HPLC/MS and MS analysis it was demonstrated that addition of β-cyclodextrin to the aqueous solution of C120 markedly inhibits the photodegradation of that dye. This results from the formation of an inclusion complex between C120 and β-cyclodextrin.

Introduction

It is well known that organic dye lasers are convenient sources of UV and visible light [1–3]. Unfortunately, the use of dyes can create some problems since dyes undergo processes such as aggregation and photooxidation which influence the efficiency of emission [1, 4, 5]. The products formed in these reactions can absorb the laser light or quench the molecules of coumarin in their excited singlet states thus decreasing the efficiency of emission. Moreover, the majority of laser dyes are poorly soluble in water. Thus organic solvents, which are environmentally unfriendly, volatile and rather expensive, have to be used.

We believe that some of these problems can be overcome by applying cyclodextrins. Cyclodextrins are cyclic oligosacharides which have the ability to include molecules of organic compounds into their cavities. Quite often the inclusion results in modification of the physicochemical properties of guest molecules [6].

In this work we present studies on the effect of α- β- and γ-cyclodextrins on the photophysical and photochemical properties of 7-amino-4-methylcoumarin (Coumarin 120), an important laser dye emitting in the blue region.

Experimental

Materials

Coumarin 120 (C120) (Sigma Chemical, Germany), α- β- and γ-cyclodextrins (all Fluka, Switzerland), acetonitrile and methanol (Aldrich Chemical, HPLC grade) were used as received.

Apparatus

UV/Vis absorption spectra were obtained using a HP 8452A diode-array spectrophotometer. Steady-state fluorescence and excitation spectra were measured using a SLM Aminco 8100 spectrofluorimeter. Excitation spectra were obtained using the front face technique. Steady-state fluorescence depolarization measurements were carried out using an SLM Aminco 8100 spectrofluorimeter equipped with Glan-Thompson calcite prism polarizers. Circular dichroism spectra were obtained using a Jasco J-710 spectropolarimeter. Mass spectra were determined using a Finningan MAT 95S sector mass spectrometer (Finnigan MAT, Bremen, Germany). Experiments were performed in EI and ESI modes. Tandem mass spectrometry: high-energy collision-induced dissociations (CID) at constant B/E, were performed in the collision cell, located in the 1st field-free region of the instrument (FFFR). Helium was used as a collision gas and the intensity of the parent ion was adjusted to approximately 30% of its initial abundance.

Irradiation of the solutions were carried out using a Rayonet photoreactor equipped with 16 lamps emitting light at 350 nm. The solutions were irradiated in air.

Quantitative and qualitative analyses of the systems studied were carried out by using a Waters HPLC system with a Waters Symmetry C18 15 μm column (3.9 $\times$ 150 mm) and equipped with a Waters 2487 Dual Absorbance Detector or diode-array detector. A mixture of water and acetonitrile (1 : 1) was employed in an isochronal mode as the mobile phase at a flow rate of 1 ml/min. For all HPLC-MS experiments a Pharmacia Biotech μ RPC C2/C18 SC 2.1/10 column was used. The column was connected to the Shimadzu LC-10AD VP gradient pump (Shimadzu, USA). The flow rate through the column was 50μl/min. The mobile phase consisted of acetonitrile and water. The gradient

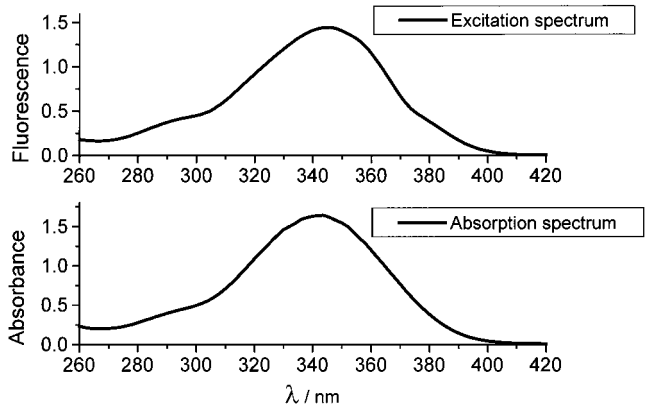


Figure 1. (A) Excitation spectrum of C120 (1×10^{-4} M) measured at the maximum of fluorescence of C120, i.e., λ = 440 nm. (B) Absorption spectrum of C120 (1×10^{-4} M).

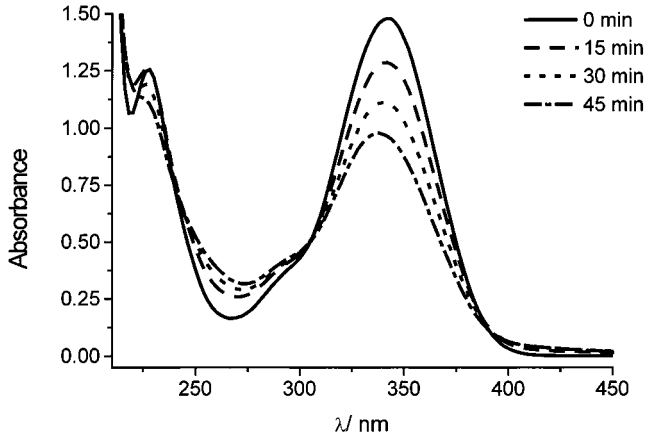


Figure 2. Absorption spectra of an aqueous solution of C120 (1×10^{-4} M) after various times of irradiation in air (0, 15, 30, 45 min).

technique (from 0 to 40% of acetonitrile over 25 min) was applied.

For the HPLC-MS experiments, the outlet of a column was directly connected to the Shimadzu SPD-M10A VP Diode Array Detector and then to the ESI MS.

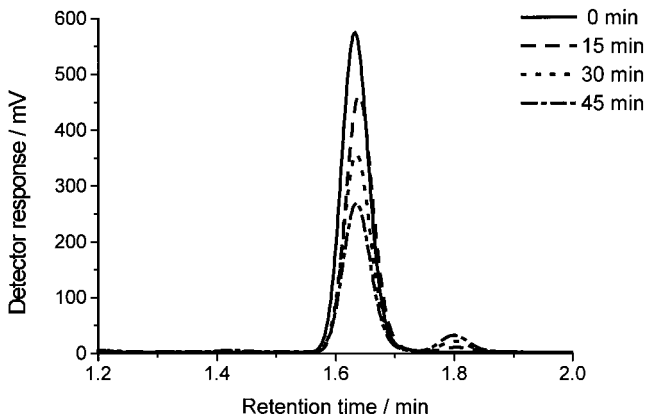
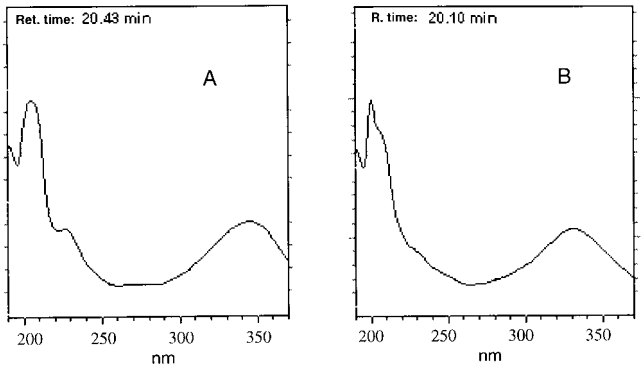


Figure 3. HPLC traces obtained for the aqueous solution of C120 (1×10^{-4} M) after various times of irradiation in air (0, 15, 30, 45 min).



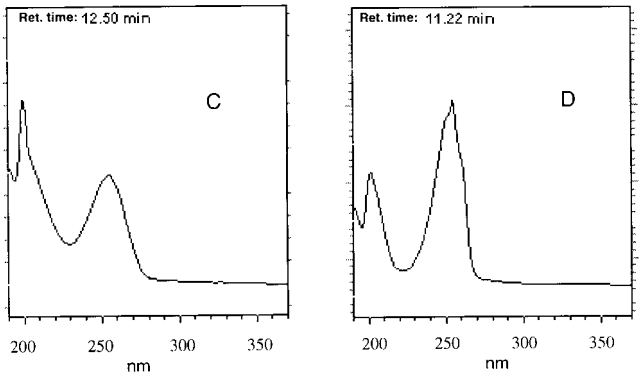


Figure 4. Results of the HPLC analysis (diode-array detection) for the aqueous solution of C120 (1×10^{-4} M) after 2 hours of irradiation: absorption spectra recorded for the unreacted C120 (A), secondary product (B), and further products (C) and (D).

Results and discussion

Aggregation

Electronic absorption and excitation spectra of C120 in aqueous solutions at several different concentrations in the range $1 \times 10^{-5} - 1 \times 10^{-4}$ M, were measured in order to determine the possible occurrence of the aggregation process. The linear dependence of the absorbance of C120, determined at the maximum of the absorption band, on concentration indicates that the Lambert- Beer law is obeyed in the system and that there is no detectable interaction between the molecules in the ground state. Also, the excitation spectrum of C 120 (even at the highest concentration used in this work, i.e., 1×10^{-4} M, collected at the $\lambda_{max.fl.}$ = 440 nm) did not exhibit any marked additional bands compared to its absorption spectrum (Figure 1). These facts indicate that in the range of concentrations studied the aggregation process of C120 in aqueous solution can be neglected.

Photochemical stability of C120

An aqueous solution of C120 (1×10^{-4} M) was irradiated in air with light at 350 nm. The reaction was followed by measurements of the absorption spectra in the UV-Vis spectral region and HPLC analysis.

Figure 2 shows the absorption spectra of an aqueous solution of C120 before and after various times of irradiation in air. One can observe that as a result of irradiation the absorption bands characteristic of C120 (λ_{max} = 240 nm and λ_{max} = 342 nm) disappear and a new absorption around 260 nm appears. There is also a small increase of the absorption around 400 nm. During the initial stage of irradiation (the first hour of irradiation) three isosbestic points at λ = 240 nm and at λ = 310 nm and at λ = 391 nm were observed. This indicates that the photochemical reaction of C120 leads to the formation of one primary photochemical product.

That fact was confirmed by the HPLC analysis. Figure 3 shows the HPLC traces obtained for the aqueous solution of C120 after various irradiation times in air. It can be seen that the irradiation results in a decrease in the concentration of C120 and the appearance of only one product. Kunjappu and Rao [7] have found that irradiation of C120 in methanol solution in the presence of oxygen leads to its oxidation resulting in formation of 7-amino-3-hydroxy-4-methyl-coumarin. It was suggested that the oxidation of C120 is a self-sensitized process and occurs with participation of singlet oxygen which is previously formed as a result of quenching of the molecule of coumarin in the excited triplet state by molecular oxygen. Our studies confirmed that during the initial period of irradiation a photoproduct of molecular mass 191, identical with that characteristic for the product found by Kunjappu and Rao [7], is indeed formed. We have found, however, that it is very difficult to perform extensive characterization of that product because it is photochemically unstable in aqueous solution. It undergoes phototransformation to the secondary product which was identified as 1-[4-amino-2-hydroxy-phenyl]-ethanone. The mass spectrometry (ESI and EI techniques) revealed that the secondary product has a molecular mass of 151. Based on the results of high resolution EI measurements the general formulas for the possible products were assigned. Additionally, the EI MS/MS analysis confirmed that the major fragmentation ion occurring at m/z = 136 can be derived from that product. Taking into account the results of both analyses it was concluded that the fragmentation of that product occurs with the loss of the —CH_3 group. This indicates that such a labile group is present in the analyzed product.

These measurements were completed with the analysis of the irradiated solutions of C120 (10^{-4} M, 3 and 9 hours) by a HPLC system equipped with a UV/Vis diode-array detector connected to the mass spectrometer in the ESI mode. That analysis allowed direct measurement of the UV/Vis spectrum of the secondary photochemical product with molecular mass of 151 (see Figure 4). The spectrum is identical to that characteristic for 1-[4-amino-2-hydroxy-phenyl]-ethanone [8]. Prolonged irradiation of the reaction mixture leads to the appearance of the further photochemical products. The HPLC analysis indicates that they appear at shorter retention times than all compounds described above and their UV absorption spectra are characteristic of substituted benzenes (see Figure 4). The above mentioned results indicate that C120 has a limited photochemical stability in aqueous solution. The suggested mechanism of photodegradation of C120 in water is shown in Scheme 1.

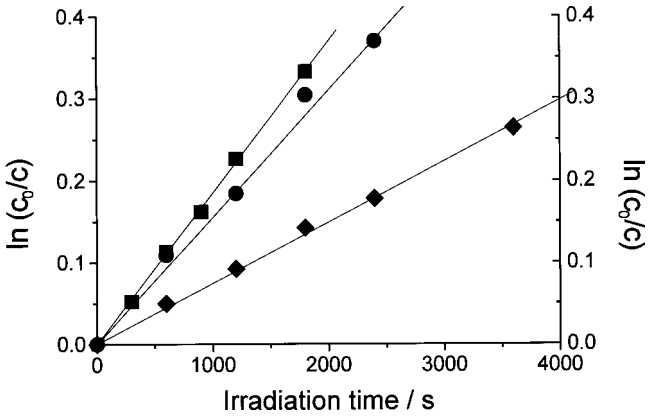


Figure 5. Kinetics of photoreaction of C120 (1×10^{-4} M) in the absence (■) and presence of cyclodextrins (all 1×10^{-2} M): β-CD (♦) and γ-CD (●).

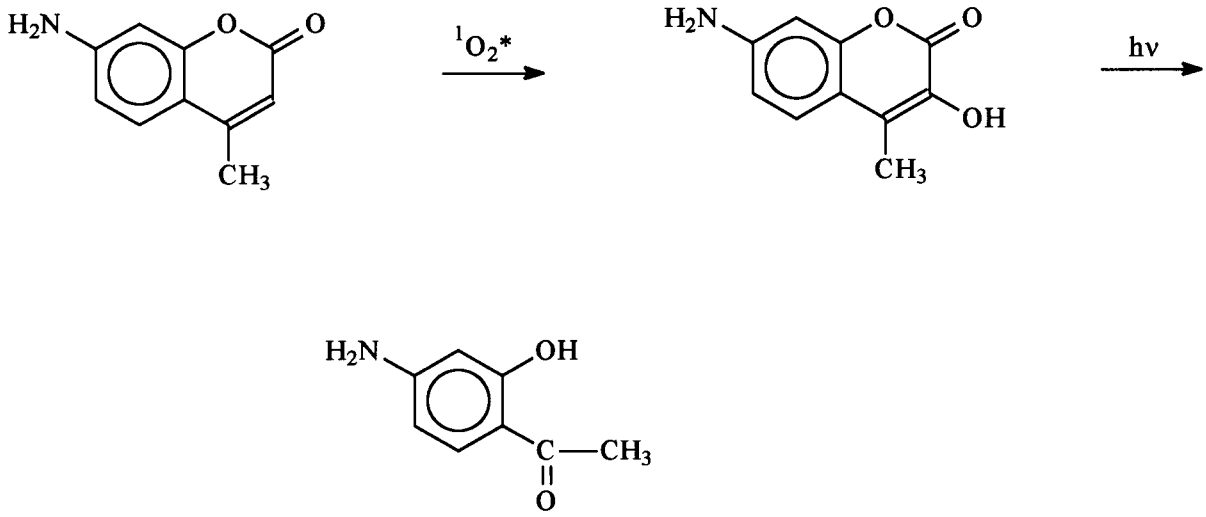
The kinetics of the photooxidation of C120 was determined using HPLC analysis. The decrease of the concentration of C120 (10^{-4} M) as a function of time was well fitted to the first order kinetic equation using a linear regression method (see Figure 5). The rate constant of photooxidation of C120 was found to be $(1.86 \pm 0.02) \times 10^{-4}$ s^{-1}.

Formation of complexes with cyclodextrins

In order to examine the possibility of formation of inclusion complexes between C120 and cyclodextrins, UV-Vis absorption spectra, fluorescence spectra and circular dichroism spectra of aqueous solutions of C120 in the presence of α-, β- or γ-cyclodextrins were measured.

The addition of α-cyclodextrin (1×10^{-2} M) to the aqueous solution of C120 (2.7×10^{-5} M) did not change the absorption spectrum of C120. On the other hand, the addition of β- and γ-cyclodextrins (both 1×10^{-2} M) induced a small decrease in the absorbance value of the C120 (stronger for β-cyclodextrin). Additionally, with β-cyclodextrin a red shift of the maximum of the C120 absorption band of about 4 nm appeared (Figure 6). This suggests the existence of interactions between C120 and β- and γ-cyclodextrin.

The measurements of fluorescence spectra have shown that addition of β-cyclodextrin resulted in a small increase in the fluorescence intensity of C120 (2.7×10^{-5} M) and the shift of the maximum of the fluorescence band by about 5 nm to the blue. There is a very small change in the fluorescence spectrum of C120 after addition of γ-cyclodextrin but no change after addition of α-cyclodextrin. These results may be well explained by the assumption that the molecule of C120 is included into the cavity of cyclodextrin. The inclusion would cause an increase in the fluorescence intensity of C120 resulting from the decrease of the intramolecular rotational freedom of the molecule in the restricted microenvironment and its protection from external quenchers [6]. On the other hand, a decrease in the fluorescence intensity of C120 induced by the change in the ambient polarity around the molecule of C120 would be observed. This is because the cavity of cyclodextrin is less polar than water and



Scheme 1. Mechanism of photodegradation of coumarin 120 in water.

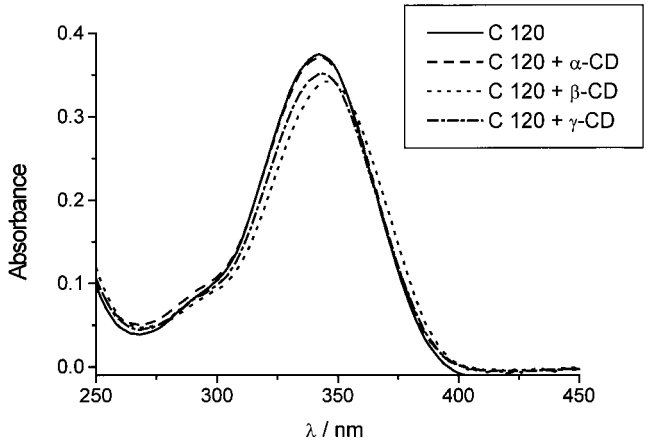


Figure 6. Absorption spectra of C120 (2.7×10^{-5} M) in the absence and presence of cyclodextrins (all 1×10^{-2} M).

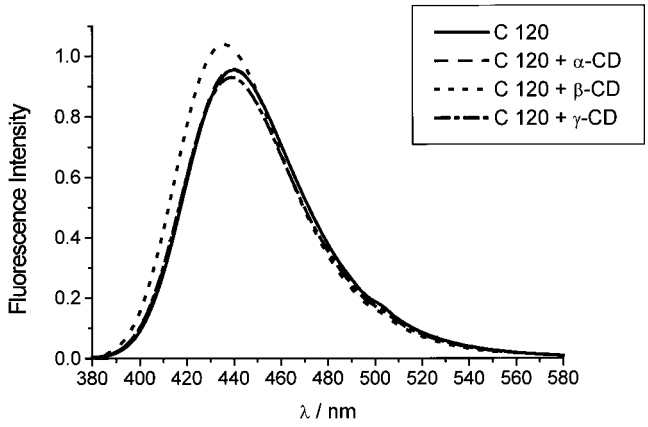


Figure 7. Fluorescence spectra of C120 (2.7×10^{-5} M) in the absence and presence of cyclodextrins (all 1×10^{-2} M).

the fluorescence intensity of coumarins is lower in apolar media [9]. Thus, eventually, a very small increase (with β-cyclodextrin) or even a decrease (with γ-cyclodextrin) in fluorescence intensity is observed (Figure 7).

The measurements of the induced circular dichroism for the aqueous solutions of C120 (8×10^{-5} M) and cyclodextrins have confirmed the existence of quite strong interactions of C120 with γ-cyclodextrin but also indicated the existence of weaker interactions between C120 and γ-cyclodextrin (Figure 8). The molecule of C120 is achiral and it does not exhibit a circular dichroism signal in aqueous solution. In the presence of β- and γ-cyclodextrins, however, induced circular dichroism (ICD) signals could be observed in the range of the absorption of C120. These have to originate from the interactions of C120 with the chiral cyclodextrins. With β-cyclodextrin the signal was about twice as strong as with γ-cyclodextrin. There is no induced circular dichroism for the C120 + α-cyclodextrin solution.

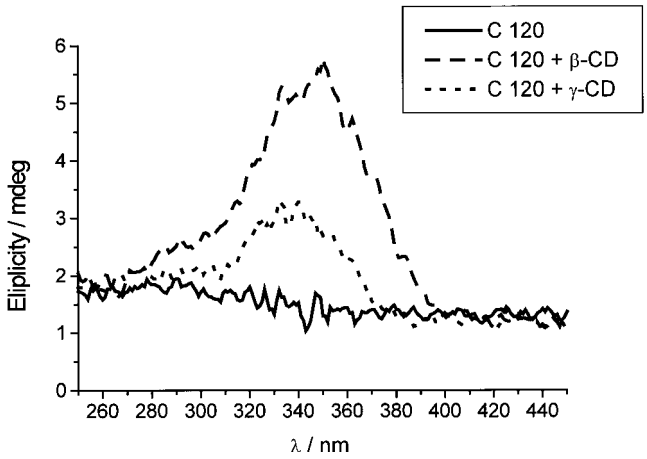


Figure 8. Circular dichroism spectra of C120 (8×10^{-5} M) in the absence and presence of cyclodextrins (all 1×10^{-2} M).

The changes in the properties of C120 in the presence of cyclodextrins, which are most pronounced in circular dichroism, can be explained considering the formation of inclusion complexes between C120 and β- and γ-cyclodextrin.

The fact of the inclusion of C120 molecules into the cavities of β- and γ cyclodextrins was confirmed by the measurements of fluorescence polarization and anisotropy for the C120 + cyclodextrins systems. The values of the polarization and anisotropy for C 120 in a presence of α-

Table 1. Values of fluorescence polarization and anisotropy of C120 alone and in the presence of cyclodextrins

System	Fluorescence polarization	Fluorescence anisotropy
C120	0.009 ± 0.001	0.0058 ± 0.0007
C120 + α-cyclodextrin	0.008 ± 0.001	0.0056 ± 0.0006
C120 + β-cyclodextrin	0.023 ± 0.002	0.0154 ± 0.0008
C120 + γ-cyclodextrin	0.012 ± 0.001	0.0079 ± 0.0007

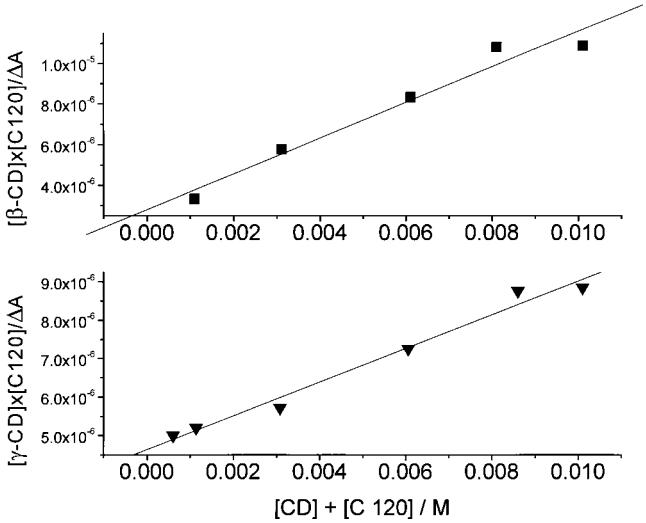


Figure 9. Results of the fit of the data to the modified Benesi–Hildebrand equation (linear regression method): for β-cyclodextrin (■) and for γ-cyclodextrin (▼).

cyclodextrin were comparable to the values obtained for C120 alone while in the presence of β- and γ-cyclodextrins the respective values were markedly higher (Table 1). Due to the inclusion of C120 into β- or γ-cyclodextrin cavities the molecules of dye experience lower rotational freedom than free molecules in solution. Thus the light emitted by such molecules while excited with polarized light is not completely depolarized. This results in higher polarization and anisotropy of the fluorescence of C120. The values of the polarization and anisotropy of the fluorescence emitted by C120 in the presence of β-cyclodextrin are about twice as large as in the presence of γ-cyclodextrin. Obviously, this indicates that the interactions of C120 with β-cyclodextrin are much stronger than those with γ-cyclodextrin.

Association constants of the complexes

The association constants for the inclusion complexes of C120 and β- and γ-cyclodextrins were calculated applying the modified Benesi–Hildebrand treatment [10] to the UV absorption and fluorescence measurements. In these experiments the concentration of C120 was 5×10^{-5} M and 1×10^{-4} M for β- and γ-cyclodextrin, respectively, and the concentration of cyclodextrins varied from 5×10^{-4} M to 1×10^{-2} M.

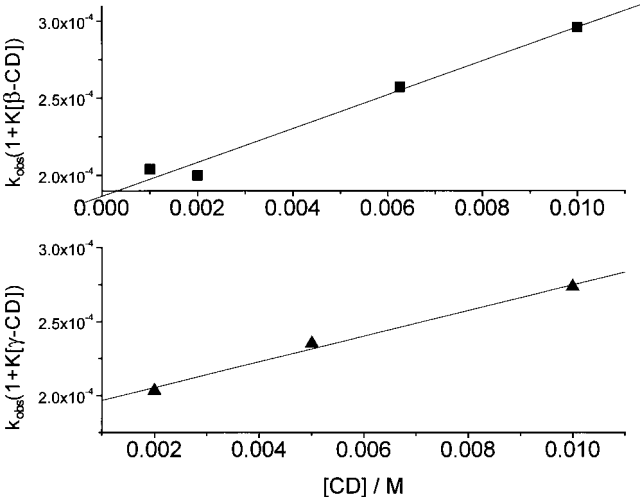


Figure 10. Dependence of the value of the left side of Equation (4) on concentration of cyclodextrin (formula used for determination of the k_2 values): for β-cyclodextrin (■) and for γ-cyclodextrin (▲).

For the calculation of association constants (K) based on the absorption spectra the Benesi–Hildebrand equation in the following form was used [10]:

$$\frac{[\mathrm{C120}]^*[\mathrm{CD}]}{\Delta A} = \frac{1}{K^*\Delta\epsilon} + \frac{1}{\Delta\epsilon}([\mathrm{C120}] + [\mathrm{CD}]), \qquad (1)$$

where [C120] is the concentration of C120, [CD] is the concentration of β- or γ-cyclodextrin, $\Delta\epsilon$ is the difference between the extinction coefficients of solutions of associated and non associated C120, ΔA is the difference between the absorbance of solutions of associated and non associated C120 (λ = 342 nm) and K is the association constant of the complex between C120 and β- or γ-cyclodextrin.

When the fluorescence spectra were used for calculation of the K value, Equation (1) was modified and used in the following form:

$$\frac{[\mathrm{C120}]^*[\mathrm{CD}]}{\Delta P} = \frac{1}{K^*k_f} + \frac{1}{k_f}([\mathrm{C120}] + [\mathrm{CD}]), \qquad (2)$$

where ΔP is the difference between the area of the fluorescence band of C120 alone and C120 in the presence of cyclodextrin, k_f is the difference of the fluorescence quantum yields of the free and the bound molecules and K is the association constant.

Experimental data were fitted to the Benesi–Hildebrand equation by using the linear regression method. In all cases the dependencies were linear (Figure 9A and B) which indicates that 1 : 1 complexes are mainly formed in the systems studied. The calculated values for the association constants (K) are collected in the Table 2. We were not successful in finding literature data for the same systems. The literature values for coumarin and β-cyclodextrin and Coumarin 460 (C460) and Coumarin 480 (C480) and γ-cyclodextrin were included in Table 2. The values are higher than those for C120 which is not surprising considering the differences in the chemical structures of these compounds.

Table 2. Values of the association constants

System	Association constant		
	UV	Fluorescence	Anisotropy
C120 + β-cyclodextrin	300 ± 100	350 ± 150	–
C120 + γ-cyclodextrin	94 ± 10	160 ± 50	–
Coumarin + β-cyclodextrin[1]	1100	1400	–
C460 + γ-cyclodextrin[2]	–	–	641
C480 + γ-cyclodextrin[2]	–	–	515

[1] *Tetrahedron* **50**, 12979 (1994).
[2] *J. Chem. Soc. Faraday Trans.* **91**, 867 (1995).

Table 3. Values of the rate constants of photodegradation of C120 alone and in the presence of cyclodextrins

System	Rate constant		
	k_{obs} ($\times 10^{-4}$ s^{-1})	k_1 ($\times 10^{-4}$ s^{-1})	k_2 ($\times 10^{-5}$ s^{-1}
C120	1.86 ± 0.02	–	–
C120 + α-cyclodextrin	1.74 ± 0.03	–	–
C120 + β-cyclodextrin	0.74 ± 0.08	1.86 ± 0.07	3.7 ± 1.6
C120 + γ-cyclodextrin	1.55 ± 0.03	1.88 ± 0.06	9.2 ± 1.8

Influence of the complexation on the photostability of C120

Aqueous solutions of C120 (1×10^{-4} M) with addition of α-, β- or γ-cyclodextrins (1×10^{-2} M) were irradiated at $\lambda = 350$ nm. The irradiated solutions were analyzed by UV-Vis spectroscopy and HPLC measurements. It was observed that cyclodextrins present in the reaction mixture influenced the rate of photochemical reactions of C120. The kinetics of the photochemical reaction of C120 (1×10^{-4} M) , both in the presence or in the absence of cyclodextrins, can be described using the first order kinetic equation (see Figure 5). The values of the experimentally determined rate constants (k_{obs}) are summarized in Table 3. Although the values of the rate constants for photooxidation of C120 in the presence of cyclodextrins were all lower than that for C120 alone, only β- and γ-cyclodextrin displayed a considerable inhibiting effect. This can be explained by considering the formation of the inclusion complexes between C120 and those cyclodextrins. The molecules of coumarin dye included in the cavity of cyclodextrin are protected against the attack of oxygen which results in the observed decrease in the rate of oxidation. Taking into account that in all systems studied there is an equilibrium between C120 associated and not associated with cyclodextrin and that the rates of reaction between C120 and oxygen are different for each of these states, the measured (observed) rate constant can be expressed as follows:

$$k_{obs} = \frac{k_1 + k_2 K[\mathrm{CD}]}{1 + K[\mathrm{CD}]}, \qquad (3)$$

where k_1 and k_2 are the rate constants for photoreaction of free C120 and C120 associated with cyclodextrin, respectively, K is the association constant and [CD] is concentration of cyclodextrin.

The rate constant (k_{obs}) for photoreaction of C120 was determined at the defined concentration of C120 (1×10^{-4} M) and various concentrations of β- or γ-cyclodextrins. (The concentration of both β- and γ-cyclodextrin changed in the range 1×10^{-3}–1×10^{-4} M). Data were plotted according to Equation (4) (Figure 10) which was obtained by transformation of Equation (3):

$$(1 + K[\mathrm{CD}])k_{obs} = k_1 + k_2 K[\mathrm{CD}]. \qquad (4)$$

The experimentally determined values of k_1 and k_2 are collected in Table 3. The fitting procedure (with linear regression method) reproduced very well the experimental value of k_1 determined experimentally in the aqueous solution without cyclodextrins. The values of k_2 are considerably lower than k_1, especially in a case of β-cyclodextrin, which reflects the better fit of C120 to the cavity of that polysaccharide.

Thus, taking into account the results of the experiments one can conclude that only β-cyclodextrin can be considered as an efficient inhibitor of the photooxidation of C120.

Conclusions

It has been demonstrated that C120 has a limited photochemical stability in aqueous solution. The addition of β-cyclodextrin to the aqueous solution of C120 markedly inhibits the photodegradation of C120. The spectroscopic experiments reveal that C120 forms a fairly stable inclusion complex with β-cyclodextrin. The rate constant of the reaction of C120 molecules included into the cyclodextrin cavity is 5 times smaller that that for uncomplexed molecules.

The influence of γ-cyclodextrin on the photostability of C120 is very limited. This may be explained by considering the lower values of the association constant between the

C120 and γ-cyclodextrin and the limited protection of the molecules complexed in the cavity of γ-cyclodextrin.

α-Cyclodextrin has nearly no influence on the photostability of C120. This is consistent with the observation that α-cyclodextrin does not form a complex with C120. Based on the experimental results one can suggest that the aqueous solution of C120 and β-cyclodextrin can be recommended as a medium for the dye laser.

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