Spectroscopy in Molecular Assemblies. Structure of Inclusion Complexes in ß-Cyclodextrin Highlighted by Fluorescent Coumarin Derivatives

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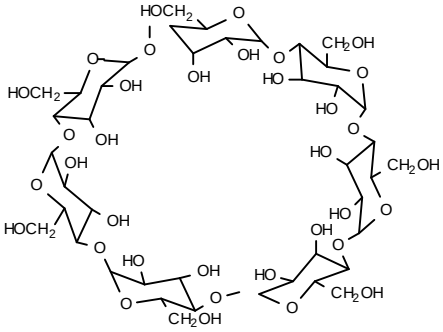
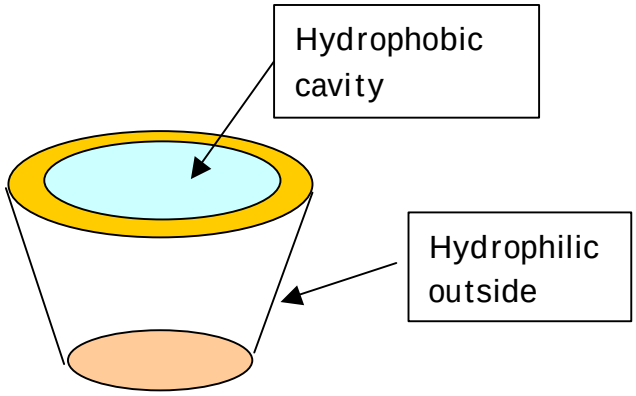
Introduction

Cyclodextrins (CD) are cyclic oligo-saccharides, obtained from starch by enzymatic degradation. They are comprised of 6, 7, or 8 glucose units,





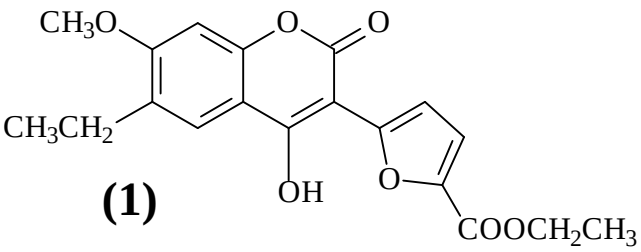
and appear like small hollow cylinders, prone to form inclusion complexes with a wide variety of guest molecules. They are excellent models of enzymes, which led to their use as catalysts, and are extensively used in chromatographic separations, electro-chemical and optical analysis. CD are also widely used in the pharmaceutical field, due to their ability to solubilize and vectorize active compounds such as retinoids, dermocorticoids and non-steroidal anti-inflammatories. They increase the chemical and photochemical stability of drugs. They can also mask undesirable side-effects, such as bad smell and irritation power.

Precise arrangements between the host and guest molecules determine the system's efficiency. A knowledge of host-guest interaction is therefore of some importance, and the incorporation of fluorescent probes within the cyclodextrin allows this investigation to be carried out by fluorimetry.[1-4]

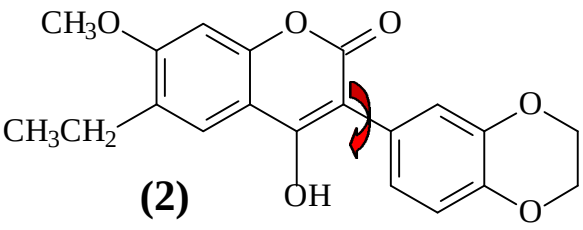
Fluorescent polysubstituted 4-hydroxy-coumarin derivatives (HCD) **1** and **2** were used in this study as models for compounds to be vectorized. It must be noted that in these compounds, both the coumaryl moieties and the heterocycle in the 3-position are virtually able to enter the CD cavity. So, for the sake of comparison, compound **3** which bears no substituent in the 3-position was studied too.

The optical properties of these probes were studied in the presence of CD. NMR was also used with the aim to relate the spectroscopic effect with the structure of the complex formed.

FLUORESCENT PROBES IN ORGANIC MEDIUM



(1)



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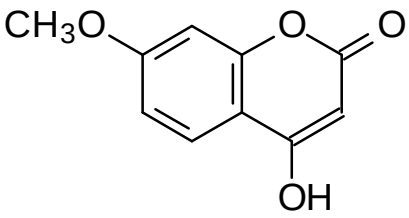


(3)

The spectroscopic properties of these dyes in organic solvents were the topic of a previous work.[5]

Probe 1

Solvent	λ_{abs}/nm	λ_{em}/nm	Φ_f	τ /ns
1,4-Dioxane	368	433	0.66	2.3
Ethyl acetate	366	429	0.79	2.2
Dichloromethane	368	425	0.17	1.1
Acetonitrile	366	427	0.38	1.8

The hydroxycoumarin derivative (HCD) **1** displayed attractive optical properties in organic medium:

- High quantum yield
- Lifetime in the ns scale
- **1** is poorly sensitive to variations of viscosity and temperature.

In contrast, HCD **2** showed

- Lower quantum yield
- Shorter lifetime than probe **1**.

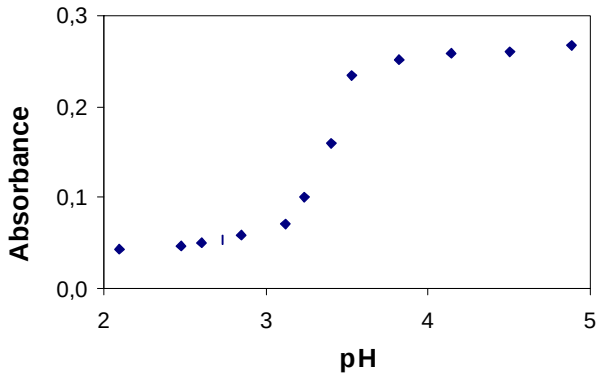
Probe 2

Solvent	λ_{abs}/nm	λ_{em}/nm	Φ_f	τ /ns
1,4-Dioxane	324	414	3.3×10^{-3}	1.0
Ethyl acetate	322	424	1.6×10^{-3}	1.2
Dichloromethane	328	414	2.3×10^{-3}	0.7
Acetonitrile	322	434	7.4×10^{-3}	≤ 0.7

The fluorescence properties of **2** are very sensitive to temperature and viscosity variations. Deactivation was attributed to the rotation of the benzodioxanyl substituent.

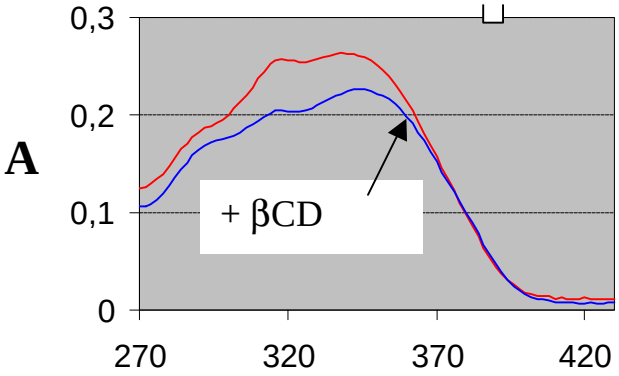
INCORPORATION IN CYCLODEXTRINS: ABSORPTION

The pKa of **1** and **2** was determined by UV/Vis absorption spectroscopy. It was found to be around 3.3. These dyes do not form aggregates in water at the concentrations used (< 10^{-5} M).



Addition of ß-CD in aqueous solutions of **1** and **2** induced a weak hypochromic shift. No effect was observed with α-CD and γ-CD.

Coumarin **1**



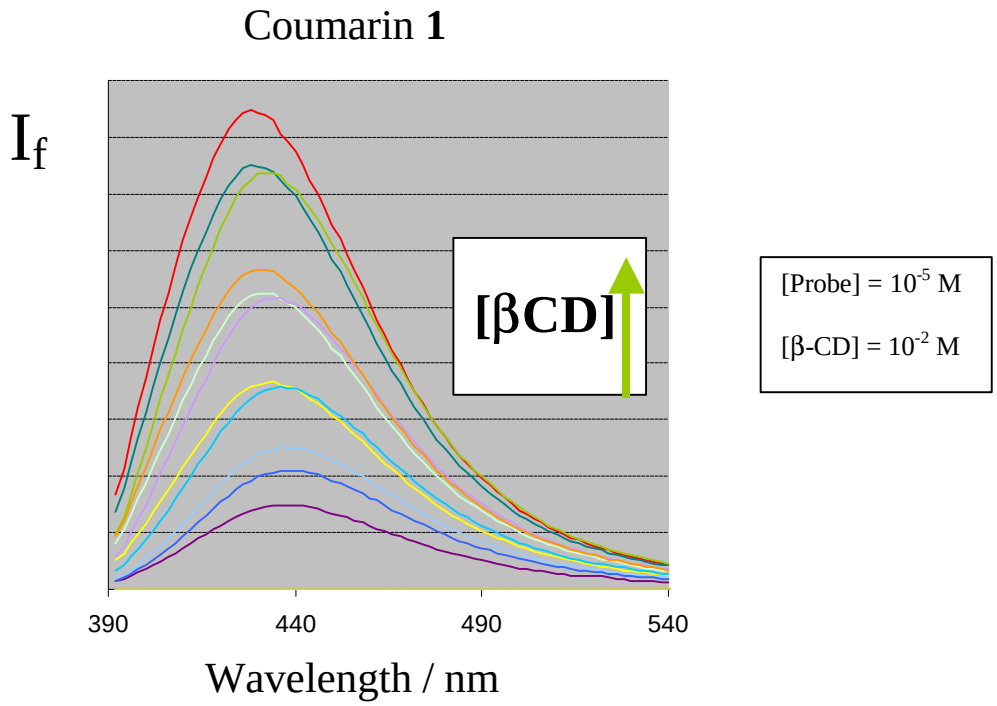
[probe] = 10^{-5} M

[β-CD] = 10^{-2} M

Wavelength / nm

FLUORESCENCE

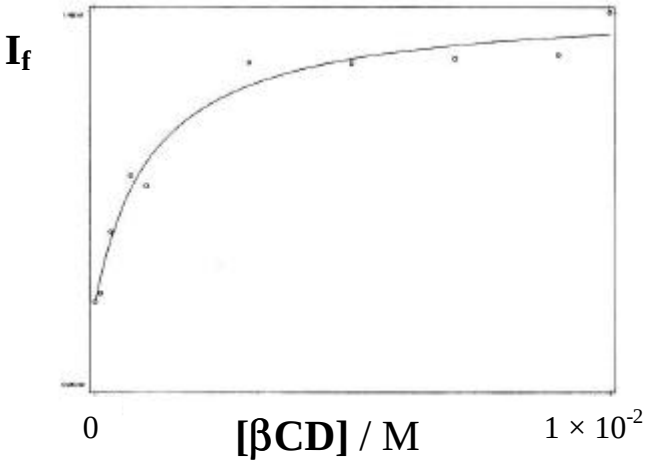
A strong increase in the fluorescence intensity was observed upon addition of ß-CD. The fluorescence quantum yield was multiplied by a factor of 6.4 for **1**, 10 for **2**, and 2.3 for **3**.



A blue-shift was also observed in the presence of ß-CD, together with an increase of the lifetime. This indicates that the dye was localized in a medium less polar than water, and that its excited state was stabilized.

DATA PROCESSING

The emission data were processed.



Points are experimental
The curve is calculated

A satisfactory fit was obtained by assuming a 1 :1 stoichiometry :

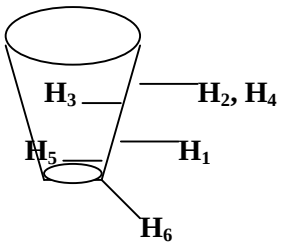
$$CD + C \rightleftharpoons CD\text{-}C \qquad K = \frac{[CD\text{-}C]}{[CD].[C]}$$

The binding constants were calculated:

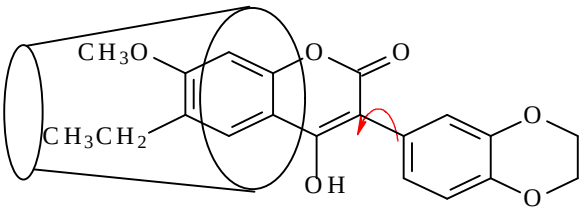
coumarin	K
1	**7×10^2**
2	**3.4×10^2**
3	**8.1×10^1**

NMR SPECTROSCOPY

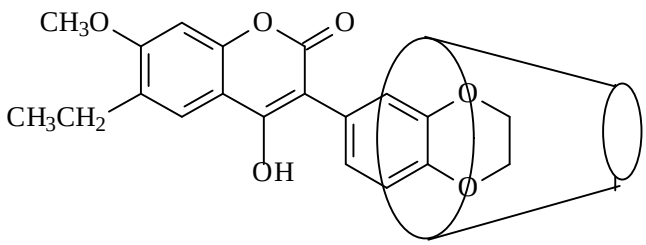
In the presence of guest, the reasonning is that upon formation of an inclusion complex, the 'inside' protons are more strongly affected than the others. Alternatively, if the interaction takes place at the exterior of the torus, the shift mainly concerns the 'outside' protons. In our case, a strong shielding effect was observed for protons H_3 and H_5. $\Delta\delta$ was very weak for the other protons. So, NMR confirms incorporation.



Concerning the guest molecule, the protons of the coumarin methoxy and ethyl groups were strongly affected. For **1** and **2**, the protons of the substituent in the 3-position, which are close to the coumaryl cycle, were also strongly shifted. Formation of complex *a* seems to be the most likely.



a



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IN SUMMARY

* The spectrophotometric study gave strong indications that an interaction takes place between the hydroxycoumarin derivatives and ß-CD. The spectral properties of HCD in the presence of ß-CD were strongly reminiscent of those obtained in organic medium, suggesting the formation of an inclusion complex. The ^{1}H NMR allowed a thorough understanding of the nature of the inclusion complex.

* Compound **3** led to complexes of low stability. However, its size was appropriate for good insertion within the CD cavity, and coumarin is known to form highly stable complexes with ß-CD. Consequently, the hydroxyl group in the 4-position has a negative influence on complex formation. In contrast, grafting a substituent on the 3-

position led to a marked increase of the binding constants for **1** and **2**.

* It was shown here that the substituent itself establishes interactions with the ß-CD. On the first hand, these interactions induced a hindrance of the substituent rotation, which was evidenced by the fluorescence behaviour of the HCD.

* Concerning the inclusion complex structure, no 1:2 complex was detected. Moreover, only one type of 1:1 complex was detected, at least for **1** and **2**, although four different complexes were expected, due to the dissymetry of the HCD and ß-CD molecules. This probably results from the presence of the substituent in the 3-position, which governs the structure of the formed inclusion complex.

APPLICATIONS AND PROSPECTS

Cyclodextrins are increasingly used for drug delivery. Their toxicity is weak, and they can vectorize compounds of biological interest. The probes used in this study are models for natural coumarin derivatives which have recently been extracted[6] from Triphasia trifolia (rutacea). This plant grows in the Caribbean, and is known in indigenous medicine for its anti-parasitic activity.

Triphasia Trifolia

Purification sometimes makes the drugs insoluble, because they are deprived of the molecules they are naturally associated with. Consequently, it is important to solubilize these drugs again.

In this study, solubilization reached 87% for **1** and 77% for **2** using 10^{-2} M ß-CD and 10^{-5} M coumarin and it was limited by the solubility of CD. However, CD's can also be chemically modified in order to increase their solubility in water.

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