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DODC Aggregation in β-Cyclodextrin Followed by Temperature-Dependent Visible Absorption and CD Spectroscopy and Singular Value Decomposition

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Abstract. The concentration and temperature dependent dimerization of 1,1′-diethyloxadicarbocyanine iodide (DODC) in aqueous solutions of β-cyclodextrin (β-CyD) is studied by UV/vis and circular dichroism (CD) spectroscopy. The spectroscopic data are analyzed by singular value decomposition and yield equilibrium constants in agreement with known values. Thermodynamic parameters are obtained and discussed. The almost constant dissymmetry factor of the induced CD over the whole experimental range is further evidence of a free monomer and complexed dimer of DODC.

Key words: β-Cyclodextrin, DODC, UV/vis spectroscopy, induced CD, singular value decomposition, thermodynamic data.

1. Introduction

The naturally occurring cyclic oligosaccharides known as cyclodextrins combine with a variety of compounds to form stable host–guest complexes, a property not only of enormous practical importance but one that has also provoked intense scientific curiosity [1]. Sato and coworkers have shown, e.g. [2], that the inherent tendency of cyanine dyes to form molecular aggregates is significantly enhanced in the presence of cyclodextrins, provided certain steric requirements are met relating to the geometric fit between the host and the guest. Their assumption of sandwich-like dye dimers stabilized inside the cyclodextrin cavity was supported by model calculations [3] and by measurements of the circular dichroism (CD) exhibited by these chiral complexes [4]. In contrast to the CD data, which allow unambiguous differentiation between complexed chiral dye aggregates and aggregates that exist in solution (and therefore do not exhibit chiroptical properties), the UV and visible spectral data of these species are so similar that more complex methods are necessary to elucidate the structures and stoichiometries of the different complexes that probably coexist in equilibrium [5]. To overcome one of the main difficulties, viz. the quantitative analysis of spectra with overlapping bands, we have applied

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a mathematical procedure (singular value decomposition or svd), which treats all the information contained in the spectra and projects out of this manifold the main components that contribute to the spectra. Equilibrium constants for the complexation of DODC in aqueous solutions of β-CyD are derived in this manner and, from their temperature dependence, the thermodynamic data are extracted. The CD measurements support the contention that the main constituents of the equilibrium under the experimental conditions are the free monomer dye and the complexed dimer dye.

2. Experimental

2.1. REAGENTS

DODC (1,1′-diethyloxadicarbocyanine iodide) was purchased from SIGMA and β-cyclodextrin (β-CyD) from Merck. Only triply distilled water was used to prepare the solutions.

2.2. APPARATUS

Visible absorption measurements were performed with a Perkin-Elmer Lambda 5, the CD measurements with a Jobin-Yvon CD6 spectrometer, both equipped for controlled temperature measurements and for data acquisition and digital handling of spectral data.

2.3. PROCEDURE

Quantitative UV/vis and CD spectral measurements of aqueous solutions containing different concentrations of DODC and β-CyD were performed, keeping the concentration of the oligosaccharide constant at 5×10^{-3} M. The dye concentration was varied between 7.5×10^{-6} and 1.0×10^{-3} M. Stock solutions of the dye were used, and complete mixing was assured by use of an ultrasonic bath. Temperature controlled (± 1 °C) measurements were performed in the range between -10 and +30 °C in steps of 10 degrees. Volume contraction of the solvent in this range amounts to less than 0.3 %, so it was neglected. Eighty spectra were obtained. Of all of these the ones at lowest temperature were not used for the data analysis due to freezing problems due to the low particle content.

3. Results and Discussion

3.1. UV/VIS DATA AND SVD RESOLUTION

A representative set of absorption spectra obtained at 30 °C and covering the full range of dye concentrations is shown in Figure 1. With increasing dye concentration there is a steady decrease of the 578 nm and an increase of the 530 nm absorption band, with a clear isosbestic point at 547 nm. For some of the highest concentrations

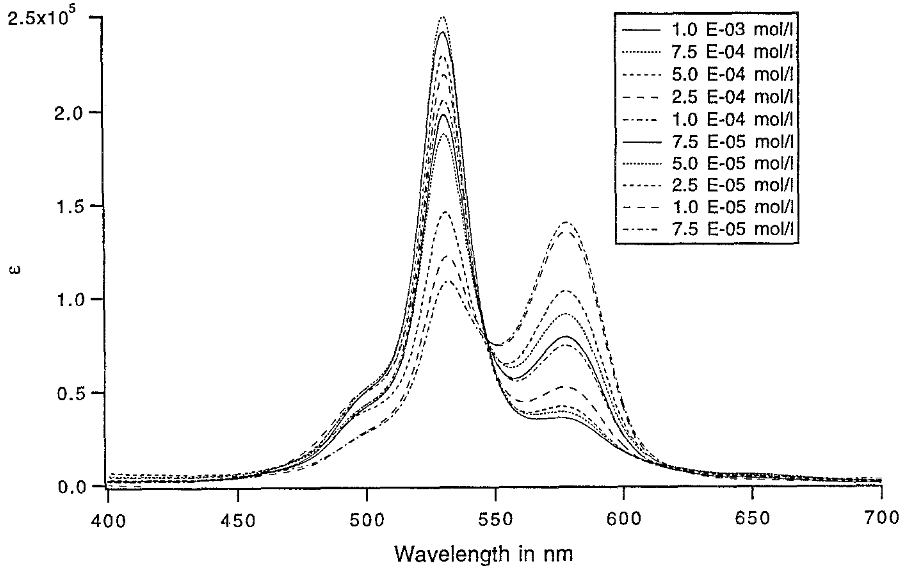


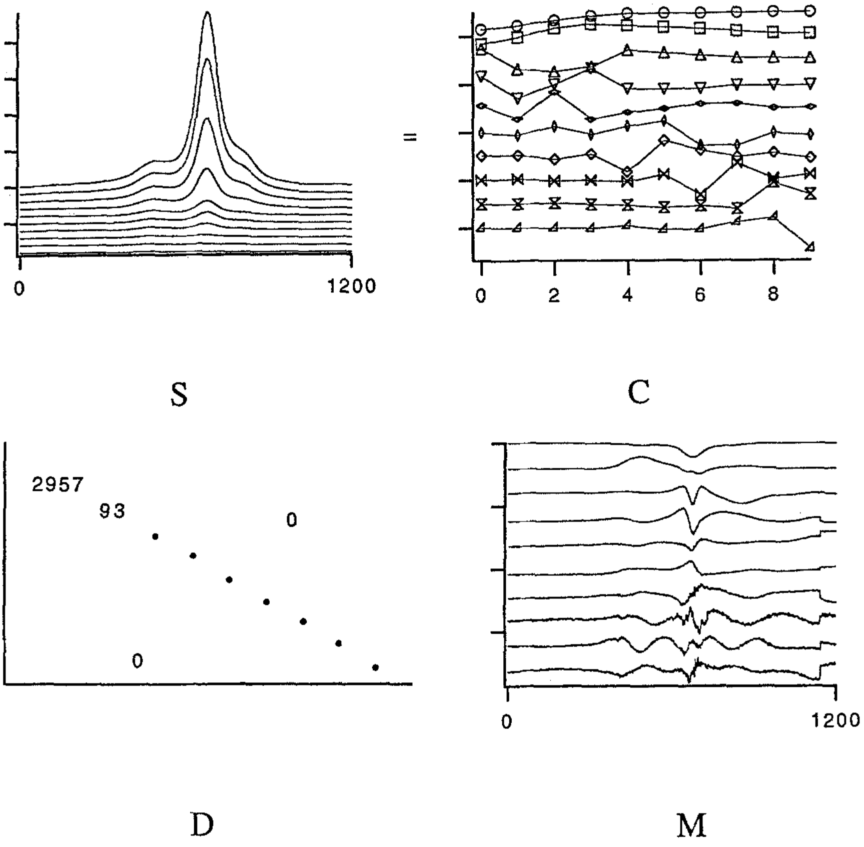
Fig. 1. Concentration dependent absorption spectra of aqueous solutions of DODC in the presence of 5×10^{-3}M β-CyD. Temperature $T = 30$ °C.

the dimer 530 nm band absorption decreased, an observation that may be caused either by higher aggregation (for which, however, we fail to see other spectroscopic evidence) or loss of dye due to precipitation at the walls of the cuvette. There is a shoulder at 500 nm and weak but distinct absorption at 650 nm.

One of the main difficulties in the quantitative analysis of these spectra is the extensive band overlap of the different components contributing to the overall spectrum. To overcome these difficulties we have applied singular value decomposition (svd) to the spectra. Svd is a mathematical tool for solving sets of linear equations that are either singular or close to singular. The theory underlying the method [6] and relevant applications are described in detail elsewhere [7].

Mathematically, a spectrum taken at k different wavelengths can be represented as a vector in k-dimensional space. A set of n spectra may be combined into a matrix **S** that is partitioned into a product of three matrices **C**, **D** and **M**, according to $\mathbf{S}_{n,k} = \mathbf{C}_{n,n} \cdot \mathbf{D}_{n,n} \cdot \mathbf{M}_{n,k}$. In this equation **M** represents a matrix containing n rows of similarity transformed vectors of dimension k; in our case $n = 8$ and $k = 1200$, i.e. each of the eight spectra taken at one temperature is represented by 1200 data points. **D** is a diagonal matrix weighing the contribution of these vectors, and the elements of the orthogonal matrix **C** are the coefficients for the combination of the vectors in **M** into the original vectors in **S**; i.e., **M** forms the basis in which the spectra are expanded. The contents of the four matrices as they appear after svd are shown in Scheme 1.

There are just two significant non-zero elements in **D**, the remaining six adding up to less than 1% of the sum of the diagonal elements. This shows that the spectra,



Scheme 1.

to a very good approximation, can be constructed as linear combinations of just two spectra, which are presumably the monomer and the dimer spectrum.

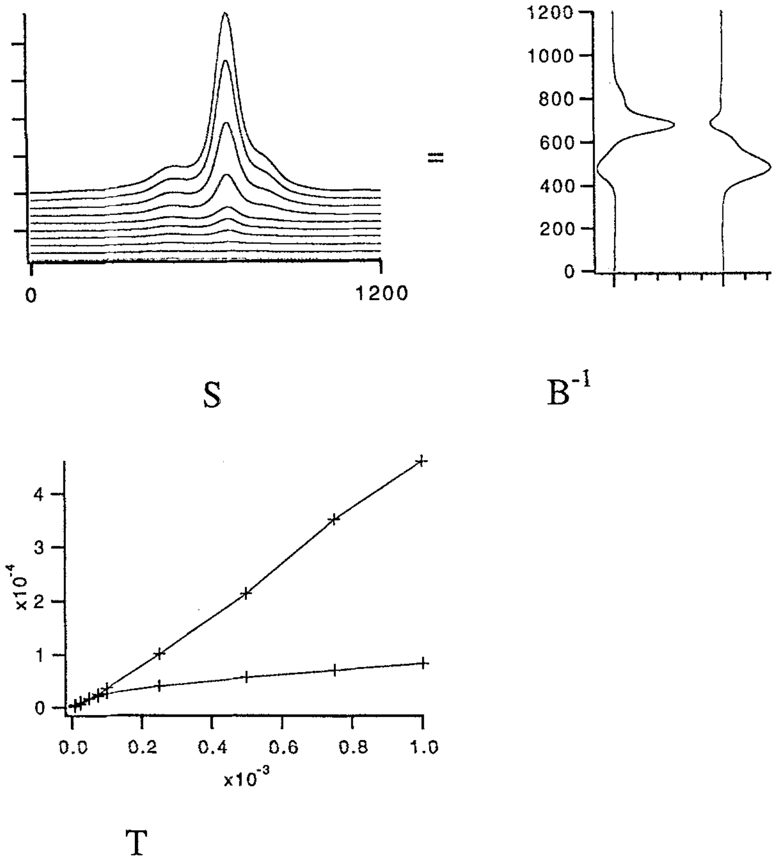
With this information a linear regression analysis is performed to obtain the best fit of the observed spectra to a two-spectrum equilibrium. To this end, a $2 \times k$ matrix **B** is constructed that contains as rows the monomer and the dimer spectra which are linearly combined to form **S**. These spectra are obtained, respectively, from the spectrum of DODC in methanol at low concentration (no aggregation) and in β-CyD at high concentration and low temperature. The product of **S** and the pseudo-inverse $\mathbf{B}^{-1}$ of **B** gives the matrix **T** which contains the concentrations of the monomer and the dimer species as a function of the overall concentration. The contents of these matrices are shown in Scheme 2.

Assuming an equilibrium

$$2\text{DODC} + \beta\text{-CyD} \rightleftharpoons (\text{DODC})_2 \cdot \beta\text{-CyD} \qquad (1)$$

with equilibrium constant

$$K = [\text{DODC}_2 \cdot \beta\text{-CyD}]/[\text{DODC}]^2[\beta\text{-CyD}], \qquad (2)$$



Scheme 2.

the constant K is fitted to the data contained in **T** such that the difference between the calculated and observed monomer/dimer distribution becomes minimal (least squares approximation). The values for K calculated in this manner are 8.3, 3.2, and 1.3×10^7 mol^{-2}L^2 at 10, 20, and 30 °C, respectively. (For comparison, the dimerization constant reported by Sato *et al.* for the same system [2] is 7×10^7 at room temperature.)

The three equilibrium constants give values for the enthalpy and entropy of the dimerization reaction of −66.5 kJ mol^{-1} and −84 J mol^{-1} K^{-1}, respectively. Other data concerning the inclusion of a dimeric host by cyclodextrin are comparable: −48 kJ mol^{-1} and −32 J mol^{-1} K^{-1}, resp., for oxacin-1 perchlorate [5]; −41 and −48 for 2-naphthalenesulfonate [8]; −56 and −47 for methyl orange [9], all relating to a γ-cyclodextrin host. The opposite signs of ΔH and $-T\Delta S$, that we observe for the DODC$_2$–β-CyD complexation is one more example of enthalpy–entropy compensation, with, however, the larger contribution of the enthalpy term dominating over the entropy loss.

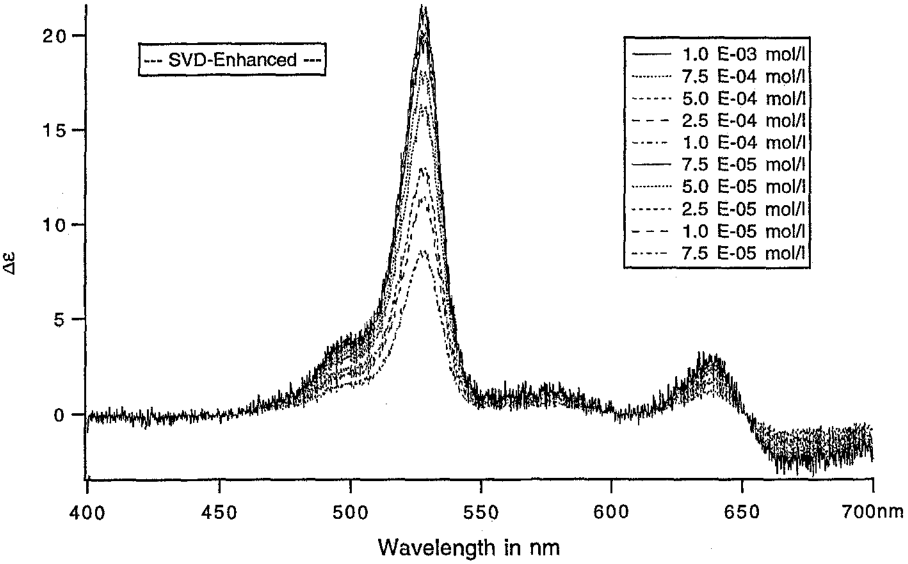


Fig. 2. Concentration-dependent CD spectra of aqueous solutions of DODC in the presence of 5×10^{-3}M β-CyD. Temperature $T = 30$ °C.

TABLE I. Dissymmetry factor $\Delta\varepsilon/\varepsilon$ ($\times 10^4$) as a function of concentration and temperature.

	0 °C	10 °C	20 °C	30 °C
10^{-3} M	1.05	0.99	1.00	0.88
10^{-4} M	1.11	1.03	1.05	0.95
10^{-5} M	1.11	1.06	0.99	0.92

4. CD Data and Structure Discussion

The CD spectra taken under identical conditions as the visible spectra in Figure 1 are shown in Figure 2. They are dominated by a positive band which is slightly blue-shifted compared to the dimer band, with a maximum at 527 nm and increasing amplitude with increasing concentration. There is a shoulder at 500 nm, and a small exciton-like absorption couplet is observed centered at 650 nm. We have no explanation for this, but it coincides with the aforementioned absorption in the visible spectrum. Note the absence of any CD at 580 nm, i.e. there is no monomer CD absorption.

We have earlier communicated our findings [4] that the enhanced aggregation of cyanine dyes in the presence of cyclodextrins is manifest not only in the visible, but also in the CD spectra of the inclusion complex. The typical exciton-split spectra observed for these species at low temperatures (down to −80 °C in 1/1 glycol/water mixtures) were interpreted in terms of twisted dimers [10] or even trimers stabilized inside the cyclodextrin pore. Those spectra are in stark contrast to the spectra observed under the relatively mild conditions described above. Disregarding the small blue shift of 3 nm of the CD maximum we observe a close coincidence between the CD and the visible dimer spectrum, both with respect to concentration and temperature dependence.

The coincidence between the UV/vis and CD data is best described in terms of the dissymmetry factor $g = \Delta\varepsilon/\varepsilon$, in which $\Delta\varepsilon$ and ε refer, respectively, to the CD maximum at 527 and the visible maximum at 530 nm. This factor is of the order of 10^{-4}, and is almost constant over the concentration and temperature range studied, as can be seen from an inspection of Table I. There is a slight concentration dependence and a small but possibly significant dependence of the g factor on the temperature, its value decreasing by about 10 to 20% in going from 0 to 30 °C which could indicate a loss of interaction between host and guest due to increased (rotational) motion.

Assuming a sandwich type dimer included axially in the cyclodextrin pore and lacking internal twisting only induced CD can be observed. From theoretical considerations [11] the positive sign of the CD absorption band is in agreement with this conclusion. Whether the different behaviour of these complexes observed under different conditions is due to the solvent used or has other reasons as well is subject to ongoing research.

5. Conclusions

Temperature and concentration dependent UV/vis and CD measurements have confirmed the preferred inclusion of DODC in β-CyD as a dimer. The absence of any exciton-split CD data indicate that the dimer is incorporated without internal twist: only the high-energy state is observed in the visible and the CD, while the low-energy state, which is forbidden under these circumstances, is not detected. The thermodynamic parameters of the equilibrium reveal that the enthalpic stabilization of the complex is partly offset by entropic destabilization.

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