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Inclusion of Rhodamine B by β-Cyclodextrin. An Equilibrium and Kinetic Spectrophotometric Study

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Abstract. A UV/visible spectrophotometric temperature-jump study of the inclusion of the rhodamine B zwitterion (RB) by β-cyclodextrin (βCD) to form a 1:1 complex (RB·βCD) in aqueous 1.00 mol dm^{-3} NaCl at pH 6.40 and 298.2 K yields: $k_1 = (1.3 \pm 0.2) \times 10^8$ dm^3 mol^{-1} s^{-1}, $k_{-1} = (2.2 \pm 0.5) \times 10^4$ s^{-1}, and $K_1 = (5.9 \pm 2.3) \times 10^3$ dm^3 mol^{-1} for the equilibrium:

$$RB + \beta CD \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RB \cdot \beta CD \qquad K_1$$

Under the same conditions the dimerization of RB:

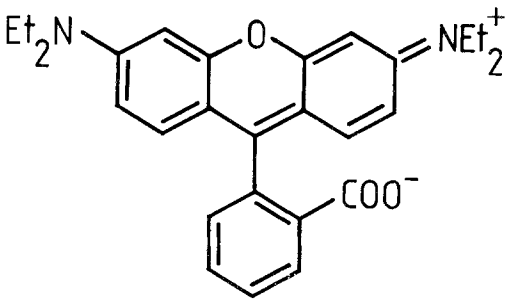
$$2\,RB \rightleftharpoons (RB)_2 \qquad K_d$$

is characterized by $K_d = (1.8 \pm 1.0) \times 10^3$ dm^3 mol^{-1}. The interaction of RB with αCD and γCD is weaker than with βCD, and is discussed in terms of the relative sizes of RB and the cyclodextrin annulus. Comparisons are made with the inclusions of other dyes by cyclodextrins.

Key words: Cyclodextrin, rhodamine B, equilibrium, kinetics, temperature-jump.

1. Introduction

The α-, β- and γ-cyclodextrins (CD) are six-, seven- and eight- membered α-1,4-linked cyclic oligomers of *D*-glucopyranose, with internal annular diameters of 4.7–5.2, 6.0–6.4 and 7.5–8.3 Å, respectively [1, 2]. The systematic variation in size of these cyclodextrins presents an ideal opportunity to study the effect of spatial relationships on both the stability and lability of inclusion complexes [1–9]. However, the majority of such studies of the cyclodextrin inclusion complexes have been thermodynamic in nature, and few kinetic studies have been reported until recently [4–9]. Several organic dyes have proven to be good probes for cyclodextrin inclusion processes, and as an extension of our studies in this area the rhodamine B zwitterion (RB) [10], whose structure is shown below, is used as a probe to investigate equilibrium, kinetic and related aspects of cyclodextrin inclusion complex formation.



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2. Experimental

Rhodamine B (Sigma) was purified by a literature method and the literature molar absorbance of 1.05×10^5 dm^3 mol^{-1} cm^{-1} at 555 nm [11] was adopted in all RB concentration determinations. The α-, β- and γ-cyclodextrins (Sigma) were stored as the anhydrous material over P_2O_5 in a vacuum desiccator prior to use. AR grade NaCl (B.D.H.) was used as the supporting electrolyte in all solutions investigated, which were prepared by weight in doubly distilled water. It was found that RB adsorbs slightly to glass surfaces but not significantly to silica surfaces. Accordingly, precautions similar to those described for the earlier crystal violet studies [7] were taken, to ensure that all surfaces had a similar history and that significant variations in the amount of RB adsorbed did not arise. All solutions were prepared immediately prior to spectrophotometric study, and exposure to light was kept to a minimum. The solution pH was 6.40 in all cases.

Visible spectra were determined in silica cells using a Zeiss DMR 10 double-beam spectrophotometer equipped with a thermostatted (±0.1 K) cell block. All spectra were run in duplicate, recorded digitally onto paper tape at 1 nm intervals over the range 450–600 nm, and were analysed using a VAX 11-780 computer. Temperature-jump spectrophotometric measurements were made at 555 nm for the RB/βCD inclusion process and RB dimerization studies, on equipment constructed in these laboratories to a design similar to that described in the literature [12]. In the study of the RB/βCD system a temperature-jump cell of optical path length 1.00 cm was used. The temperature jump was 8.8 K and the heating time was *ca.* 2 μs. To study the dimerization of RB, a cell of optical path length 0.23 cm was used (which permitted studies of higher RB concentrations than was possible with the 1 cm path length cell) for which the temperature jump was 9.7 K and the heating time was *ca.* 5 μs. In all cases the observation temperature was 298.2 ± 0.1 K. Photomultiplier voltages from each transient were collected as 4096 8-bit data points using a Data Lab DL910 transient recorder and stored on magnetic tape. At least six transients were collected for each solution and the stored transients were averaged and subjected to kinetic analysis using a Computer Products Spectrum II minicomputer.

Circular dichroic measurements were made on a Jasco J-40CS spectropolarimeter interfaced with an Intel SDK-8085 microcomputer.

3. Results

3.1. EQUILIBRIUM AND SPECTROSCOPIC ASPECTS

Rhodamine B exists as the zwitterion [10] shown above under the conditions of this study (aqueous 1.00 mol dm^{-3} NaCl solution at pH 6.4 and 298.2 K), and its spectrum shows no significant variation in the presence of αCD. This is in contrast to the RB/βCD system where the RB spectrum varies with increasing βCD concentration (Figure 1) consistent with the formation of the 1 : 1 inclusion complex, RB·βCD:

$$\mathrm{RB} + \beta\mathrm{CD} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{RB}\cdot\beta\mathrm{CD} \qquad K_1 \tag{1}$$

A non-linear least squares fit of the molar absorbance data in the ranges 500–535 and 549–599 nm at 1 nm intervals to the standard equation for the variation of molar absorbance anticipated for equilibrium (1), in which the RB spectrum was assumed

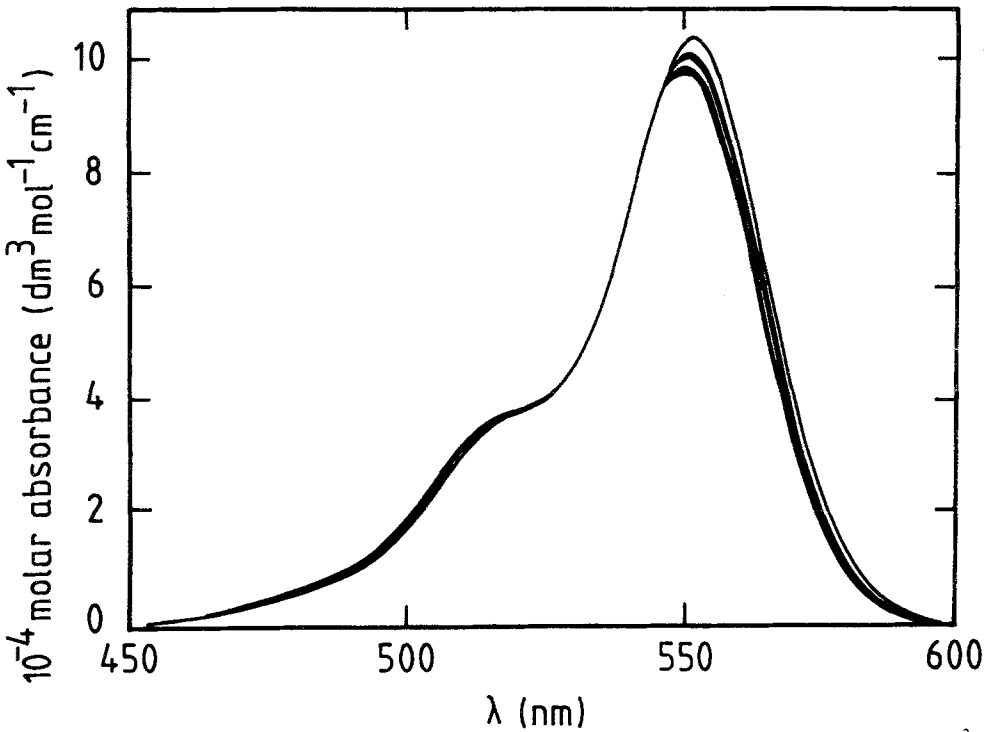

Fig. 1. Variation of the RB spectrum with total βCD concentration, [βCD], in aqueous 1.00 mol dm^{-3} NaCl at pH 6.40 and 298.2 K. The molar absorbance at 550 nm decreases systematically as [βCD] increases sequentially in the range: 0, 1.01×10^{-4}, 2.02×10^{-4}, 4.97×10^{-4}, 1.01×10^{-3}, and 5.01×10^{-3} mol dm^{-3}. Total [RB] = 9.3×10^{-6}. These six spectra exemplify the variation observed for the sixteen solutions studied in the [βCD] range: 0–1.00×10^{-2} dm^3 mol^{-1}.

identical to that observed in the absence of βCD (only *ca.* 3% of RB is dimerized under the experimental conditions as is shown below, and has a negligible effect on this assumption) and the RB·βCD spectrum was allowed to vary, yields $K_1 = (6.9 \pm 0.7) \times 10^3$ dm^3 mol^{-1}. (The best-fit spectrum of RB·βCD is similar to that of the most concentrated βCD solution shown in Figure 1, and has a maximum molar absorbance of 9.8×10^4 dm^3 mol^{-1} cm^{-1} at 553 nm.) Under the same conditions a similar, but smaller, spectral variation was observed in the presence of γCD and a fit of the molar absorbance data in the range 550–565 nm at 1 nm intervals to an equilibrium analogous to (1) yielded $K_1 = 71 \pm 18$ dm^3 mol^{-1}.

Inclusion in the cyclodextrin annulus should induce chirality in RB as evidenced by the observation of a circular dichroic (c.d.) spectrum. The c.d. spectrum shown in Figure 2 was observed under conditions where RB is predominantly included in the RB·βCD complex, and is seen to be of positive amplitude. This positive amplitude is consistent with the electronic transition moment axis for included RB subtending an angle of $\leqslant 30°$ with the principal rotational axis of βCD [13]. As the transition moment axis of RB is coincident with the long axis of its xanthene moiety [11], it follows that the major orientation of RB in RB·βCD allows the $-NEt_2$ group of RB to intrude into the βCD annulus, such that the long axis of the xanthene moiety lies close to the principal rotational axis passing longitudinally through the βCD annulus.

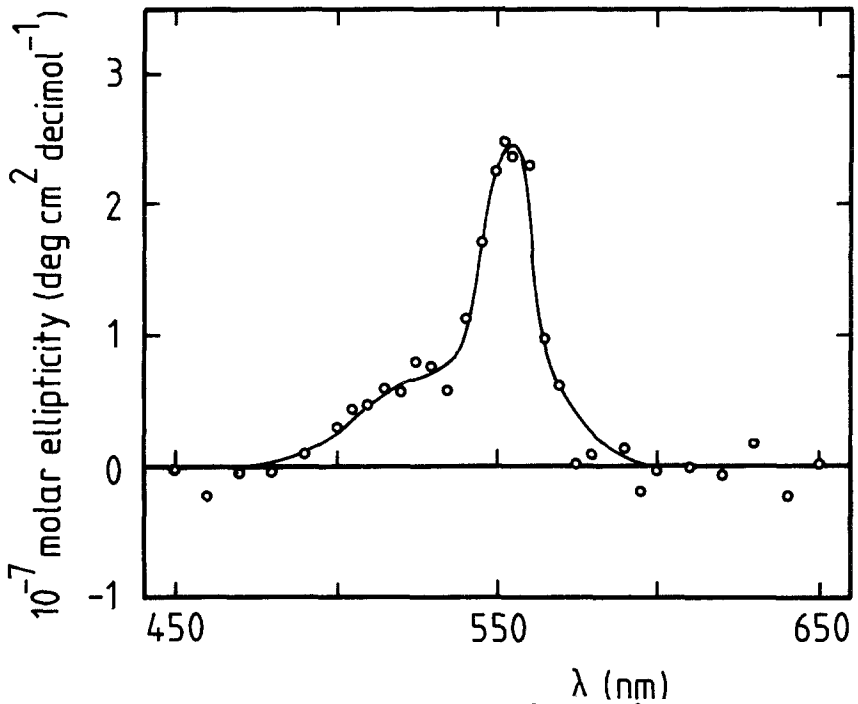

Fig. 2. Induced circular dichroic spectrum of RB (1.5×10^{-5} mol dm^{-3}) in the presence of βCD (9.7×10^{-3} mol dm^{-3}) in aqueous 1.00 mol dm^{-3} NaCl at pH 6.40 and 298.2 K.

3.2. KINETIC ASPECTS

Temperature-jump spectrophotometric studies of the RB/βCD system at 555 nm in aqueous 1.00 mol dm^{-3} NaCl at pH = 6.40 and 298.2 K detected a single relaxation characterized by an increase in absorbance. At a constant total RB concentration of 9.3×10^{-6} mol dm^{-3} the relaxation time, τ, decreased with total βCD concentration in the range $9.9 \times 10^{-5} - 6.00 \times 10^{-4}$ mol dm^{-3} as shown in Figure 3. This observation is consistent with the relaxation arising from equilibrium (1) adjusting to the higher temperature such that the variation of τ is given by Equation (2):

$$1/\tau = k_1([\mathrm{RB}] + [\beta\mathrm{CD}]) + k_{-1} \tag{2}$$

where the concentrations are the equilibrium values at the new temperature [14, 15]. A linear least-squares fit of the $1/\tau$ data to Equation 2 yielded: $k_1 = (1.3 \pm 0.2) \times 10^8$ dm^3 mol^{-1} s^{-1}, $k_{-1} = (2.2 \pm 0.5) \times 10^4$ s^{-1}, and $K_1 (= k_1/k_{-1}) = (5.9 \pm 2.3) \times 10^3$ dm^3 mol^{-1}. The good agreement between the K_1 values independently derived from the temperature-jump and equilibrium spectrophotometric data confirms the plausibility of the identification of the observed spectral variations.

A small rapid relaxation occurring within the instrumental heating time was observed in these studies and is attributed to the dimerization of RB as discussed in the next section. The spectral changes characterizing the RB/γCD system were too small for quantitative study by temperature-jump methods.

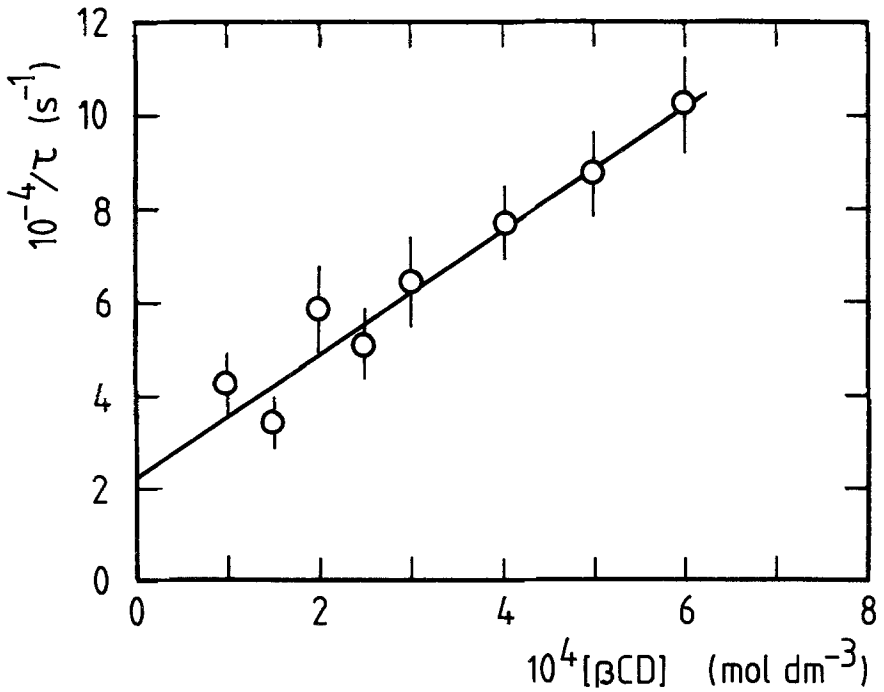

Fig. 3. Variation of $1/\tau$ characterizing the RB/βCD system obtained from temperature-jump spectrophotometric measurements in aqueous 1.00 mol dm^{-3} NaCl at *p*H 6.40 and 298.2 K. The solid line represents the linear least-squares best fit of the data to Equation 2.

3.3. DIMERIZATION OF RHODAMINE B

Rhodamine B in the concentration range $(2.81–8.00) \times 10^{-5}$ mol dm^{-3}, in aqueous 1.00 mol dm^{-3} NaCl at pH 6.40 and 298.2 K exhibits a relaxation producing an increase and a decrease in absorbance at 553 and 522 nm, respectively, which occur within the heating time of the temperature-jump instrument (*ca.* 5 μs). This relaxation is attributed to the dimerization equilibrium (3),

$$2\,\mathrm{RB} \rightleftarrows (\mathrm{RB})_2 \qquad K_d \tag{3}$$

but, because the relaxation occurs within the instrumental heating time no quantitative kinetic data can be derived for this fast process. Nevertheless, K_d may be derived from the concentration dependence of the relaxation amplitude, ΔI, at 553 nm (where the greatest change in molar absorbance occurs) through Equation (4),

$$\Delta I/I_0 = C/(4/[\mathrm{RB}] + 1/[(\mathrm{RB})_2]) \tag{4}$$

where I_0 is the detected light intensity prior to the temperature-jump and C is a constant characterizing the dimerization equilibrium [16]. The variation of $\Delta I/I_0$ with the total RB concentration is shown in Figure 4, as is the best fit of these data to Equation 4 obtained through a non-linear least-squares analysis which yielded $K_d = (1.8 \pm 1.0) \times 10^3$ dm^3 mol^{-1}. On this basis it is calculated that *ca.* 3% of the total RB concentration was present as the dimer under the conditions of the RB·βCD equilibrium and kinetic studies discussed in the preceding sections.

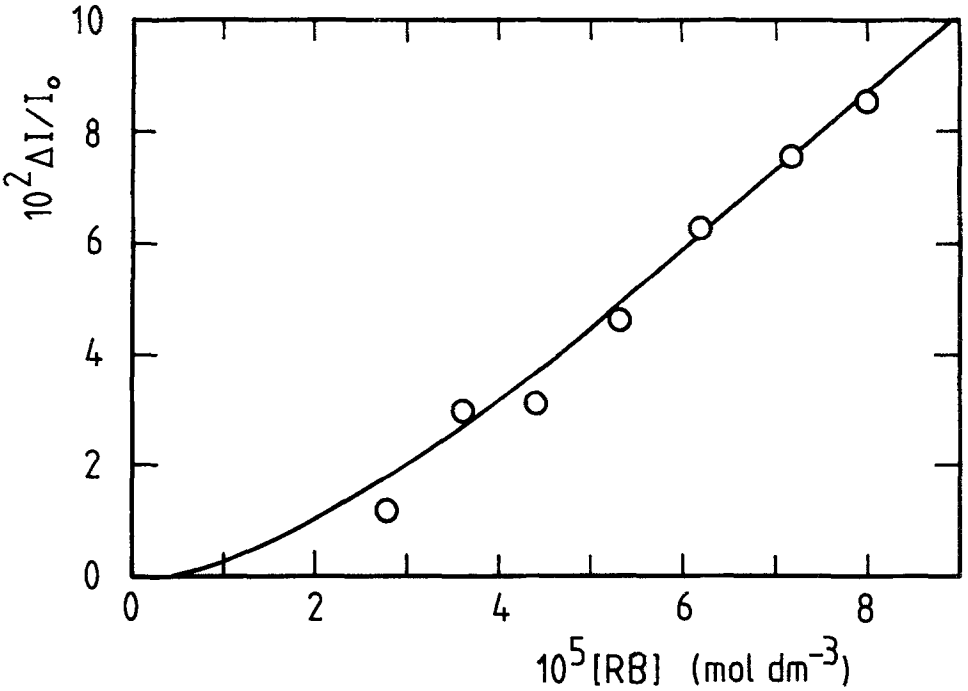

Fig. 4. The variation of $\Delta I/I_0$ with total RB concentration obtained from temperature-jump measurements in aqueous 1.00 mol dm^{-3} NaCl at *p*H 6.40 and 298.2 K. The solid curve represents the non-linear least-squares fit of the data to Equation 4.

4. Discussion

The data obtained in this study indicate that in the series α-, β-, and γCD the second cyclodextrin is of the optimal size to include RB in a 1 : 1 complex while αCD and γCD are, respectively, too small and too large to confer high stabilities on such complexes. However, before exploring these size relationships it is appropriate to compare RB with the closely related xanthene dye pyronine B (PB), which is obtained by replacing the carboxyphenyl group of RB with a hydrogen; and pyronine Y (PY), which is obtained by replacing the NEt_2 groups of PB with —NMe_2 groups. While the xanthene moieties in all three dyes are planar, the plane of the carboxyphenyl group of RB is perpendicular to the plane of the xanthene moiety [17] and this is expected to cause the geometry of the RB dimer [18] to differ substantially from the less sterically hindered PB and PY dimers. Some evidence for the effect of steric hindrance on dimer stability may be adduced from the observation that in aqueous 1.00 mol dm^{-3} NaCl at 298.2 K $K_d = (1.8 \pm 1.0) \times 10^3$ dm^3 mol^{-1} for the dimer of the overall zero charged RB zwitterion, whereas $K_d = (1.3 \pm 0.5) \times 10^3$ and $(1.1 \pm 0.2) \times 0.2) \times 10^3$ dm^3 mol^{-1}, respectively, for the dimers formed by the monocations of PB [9] and PY [19], which are expected to be less stable as a result of mutual charge repulsion.

Both RB and PY form 1 : 1 complexes with βCD characterized by parameters of similar magnitude as seen in Table I. Thus for this particular interaction the differences in structure and charge of the dyes do not cause major differences in the over-

Table I. Dye-cyclodextrin inclusion complex equilibrium and kinetic parameters (298.2 K)

Dye	$K_1/10^2$ dm^3 mol^{-1}	$K_2/10^5$ dm^3 mol^{-1}	$k_1/10^9$ dm^3 mol^{-1} s^{-1}	$k_{-1}/10^3$ s^{-1}
		β-cyclodextrin		
rhodamine B[a]	59 ± 23	–	0.13 ± 0.02	22 ± 5
pyronine B[b]	73 ± 31	–	0.11 ± 0.01	15 ± 5
Dye	$K_1/10^2$ dm^3 mol^{-1}	$K_2/10^5$ dm^3 mol^{-1}	$k_2/10^9$ dm^3 mol^{-1} s^{-1}	$k_{-2}/10^3$ s^{-1}
		γ-cyclodextrin		
rhodamine B[a]	0.71 ± 0.18	–	–	–
pyronine B[b]	4.3 ± 0.1	1.28 ± 0.04	0.82 ± 0.02	6.40 ± 0.05

[a] This work. [b] Reference [9].

all equilibrium characteristics. A dramatic difference occurs in their interactions with γCD. While RB forms RB·γCD of moderate stability, PB forms both PB·γCD and $(PB)_2 \cdot \gamma CD$ (where in the latter complex PB is included as a dimer) as shown in equilibria (5) and (6),

$$PB + \gamma CD \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} PB \cdot \gamma CD \qquad K_1 \tag{5}$$

$$PB + PB \cdot \gamma CD \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} (PB)_2 \cdot \gamma CD \qquad K_2 \tag{6}$$

which are characterized by the parameters in Table I. The $(PB)_2$ dimer is stabilized by a factor of 100 through inclusion in $(PB)_2 \cdot \gamma CD$, whereas the analogous $(RB)_2$ complex was not detected in this study. In view of the similar K_d values characterizing $(PB)_2$ and $(RB)_2$ in the absence of cyclodextrins, it is concluded that the alignment of the plane of the carboxyphenyl group perpendicular to the plane of the xanthene moiety in RB not only has a major influence on the structure of $(RB)_2$, but also inhibits the inclusion of this dimer by γCD. Clearly, if RB is included by cyclodextrins and $(PB)_2$ is not, the presence of βCD in particular will decrease the proportion of $(PB)_2$ in solution. This is an important consideration in the design of dye laser systems using rhodamine dyes where dimerization internally quenchs the dye fluorescence and prevents effective lasing [20]. However, the employment of cyclodextrins to suppress dye dimerization in such dye laser systems will depend upon the relative importances of equilibria analogous to 3, 5, and 6 above.

Acknowledgements

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References

1. W. Saenger: *Angew. Chem., Int. Ed. Engl.* **19**, 344 (1980).
2. M. L. Bender and M. Komiyama: *Cyclodextrin Chemistry* Springer, Berlin (1978).
3. J. Szejtli: *Cyclodextrins and Their Inclusion Complexes* Akadémiai Kiado, Budapest (1982).

4. F. Cramer, W. Saenger, and H-Ch. Spatz: *J. Am. Chem. Soc.* **89**, 14 (1967).
5. A. Hersey and B. H. Robinson: *J. Chem. Soc., Faraday Trans. 1* **80**, 2039 (1984).
6. R. J. Clarke, J. H. Coates, and S. F. Lincoln: *Carbohydrate Res.* **127**, 181 (1984).
7. R. L. Schiller, J. H. Coates and S. F. Lincoln: *J. Chem. Soc., Faraday Trans. 1* **80**, 1257 (1984).
8. R. J. Clarke, J. H. Coates, and S. F. Lincoln: *J. Chem. Soc., Faraday Trans. 1* **80**, 3119 (1984).
9. R. L. Schiller, S. F. Lincoln, and J. H. Coates: *J. Chem. Soc., Faraday Trans. 1* **82**, 2123 (1986). In this paper all references to the cyclodextrin annular radius should be replaced by the cyclodextrin annular diameter.
10. R. W. Ramette and E. B. Sandell: *J. Am. Chem. Soc.* **78**, 4872 (1956).
11. M. E. Gal, G. R. Kelly, and T. Kurucsev: *J. Chem. Soc., Faraday Trans. 2* **69**, 395 (1973).
12. G. G. Hammes: *Investigations of Rates and Mechanisms of Reactions, Part 2* (Techniques of Chemistry v. 6, Ed. G. G. Hammes), pp. 147–185. Wiley, New York (1974).
13. M. Kajtar, C. Horvath-Toro, E. Kuthi, and J. Szejtli: *Acta Chim. Acad. Sci. Hung.* **110**, 327 (1982).
14. C. F. Bernasconi: *Relaxation Kinetics*, Academic Press, New York (1976).
15. G. H. Czerlinski: *Chemical Relaxation*, Marcel Dekker, New York (1966).
16. D. Thusius, *J. Am. Chem. Soc.* **94**, 356 (1972).
17. K. Kemnitz, N. Tamai, I. Yamazaki, N. Nakashima, and K. Yoshihara: *J. Phys. Chem.* **90**, 5094 (1986).
18. K. H. Drexhage: *Structures and Properties of Dye Lasers* (Topics in Applied Physics v. 1, Ed. F. P. Schafer), pp. 144–193. Springer, Berlin (1973).
19. R. L. Schiller, S. F. Lincoln, and J. H. Coates: submitted for publication.
20. Y. Degani, I. Willner, and Y. Haas: *Chem. Phys. Lett.* **104**, 496 (1984).