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Supramolecular Control of Photophysical Properties of Cyanine Dyes

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Abstract: We present the results of our studies on the intramolecular complexation of cyanine dyes and cyclodextrins with the objective of modifying the photophysical behaviour of the dyes. We demonstrate that dye inclusion complex formation with cyclodextrins serves to inhibit dimer formation as well as enhance photostability of cyanines. We also show that the modification of the physicochemical properties of cyanine dyes by cyclodextrin complexation is a highly selective, structure dependent phenomenon.
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INTRODUCTION

Cyanine dyes[1] are a class of compounds for which extensive applications in photography, optical recording technology[2] and fluorescent detection[3] of analytes in diagnostics have been demonstrated. They are highly fluorescent and generally absorb and emit light in the 400 - 800 nm wavelength region. Cyanines are a small group within a much larger class of compounds known as polymethine dyes. These dyes are characterized by the presence of a conjugated olefin terminated at each end by a heterocyclic moiety, one of which behaves as an electron donor and the other as an electron acceptor.

Despite the popularity of cyanines as fluorescent probes, there are several practical considerations that limit efficient use of these dyes. Photodecomposition and self aggregation are common phenomena associated with cyanine dyes when they are utilized at high concentrations in aqueous media.[4] Such processes can lead to diminished fluorescence intensity resulting in inconsistent interpreteration of bioanalytical data. Cyanine dyes are especially prone to forming H-dimers in concentrated aqueous solutions resulting in deviation from Beer's law. Such a deviation leads to a blue shifted absorption maximum as well as fluorescence quenching.[5] Since the photophysical behaviour of cyanine dyes is particularly sensitive to local environment effects, chemical additives that can provide suitable host microenvironments have sometimes been used when employing these dyes for fluorescence analysis. Such agents include tertiary ammonium salts, organic solvents and anionic surfactants.[6] The photophysical and photochemical properties of organic fluorescent molecules can also be modified by the microenvironment induced by complexation with cyclodextrins. The observed changes resulting from cyclodextrin complexation include emission intensity enhancement,[7] fluorescence quenching,[8] excimer formation, [9] and the enhancement of lifetime of the excited state.[10] Although fluorescence quenching effects as a consequence of dye aggregation can sometimes be avoided by appropriate buffer formulation, signal loss due to photobleaching can be a major problem when using cyanine dyes for bioanalytical applications. In fluorescence microscopy, for example, an intense beam of light incident on the sample contributes to significant

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photobleaching of even relatively photostable dyes such as fluorescein and rhodamine. Although it has been shown that high concentrations of the antioxidant N-propyl gallate was efficient in retarding photobleaching, the same additive also led to fluorescence quenching when used in higher concentrations.[11]

EXPERIMENTAL SECTION

2-Methylbenzothiazole, 2-methylbenzoxazole, 2,3,3-trimethylindolinine and triethylorthoformate were purchased from Aldrich Chemical Co (Milwaukee, WI). 1,1,3-Trimethoxypropene was purchased from Kodak Chemical Co (Rochester, NY). 2,6-Dimethyl-β-cyclodextrin (Me-β-CD) was obtained from American Maize Company (Hammond , IN). All chemicals were used without further purification. Absorption spectra were obtained using a Hewlett Packard Model 8452A spectrophotometer. The quartenary and betaine salts of the heterocycles were prepared using known methods.[12a] High resolution mass spectra were obtained on a Finnigan FTICR Mass Spectrometer. Mass spectra of the N-methylated dyes were obtained in the positive ion mode, while that of the N-sulfopropyl dyes was conducted in the negative ion mode. Absorption spectra were obtained as follows: the concentrations of the stock solutions were chosen in such a manner as to ensure partial dimerization of the dye under aqueous conditions. A known amount of dye was dissolved in DMSO and further diluted with deionized water to result in 1 x 10^{-3} M aqueous solutions of dye containing 1-3% DMSO. The stock solutions were further diluted to appropriate concentrations. A 180 mM stock solution of 2,6-dimethylcyclodextrin was made in distilled water. For spectrophotometric studies of dyes, cyclodextrin concentration was chosen such that equilibrium was maintained towards dye-CD complex formation. Photobleaching of the dyes was monitored by plotting absorbance as a function of time. The dye solutions were left to stand at 25 °C under ambient light for the photostability studies.

Synthesis of 3,3'-Dimethylthiacarbocyanine iodide (**2**): The following procedure was adopted for the preparation of all the dyes.[12b,c] A mixture of 2,3-dimethylbezothiazolium iodide (0.25 gm, 0.85 mmoles), triethylorthoformate (.05 mL, 2.55 mmoles) and pyridine (1 mL) is refluxed for 4 h. Pyridine is evaporated under vaccum and the resulting dark residue is dissolved in 5 mL of methanol. Addition of 50 mL of diethyl ether is followed by cooling at -20°C overnight. The resulting dark precipitate is filtered and dried under vaccum to result in 202 mg (50 % yield) of a dark purple solid. High resolution Mass Spec data. Mol. Formula $C_{19}H_{17}N_2S_2$ Calc. Mol wt. 337.0828, found 337.0828. 1,3,3-Trimethoxypropene is substituted for triethylorthoformate to prepare dicarbocyanines.[12d] Dyes synthesized from betaine salts were further recrystallized from 60% aqueous ethanol.

RESULTS

Cyanine Dye Deaggregation

It has been demonstrated that γ-cyclodextrin-cyanine dye (**Figure 1**, Dye **1**) complexation leads to enhancement of dimerization.[13] In our laboratories we observed that heptakis 2,6-O-methyl-β-cyclodextrin (Me-β-CD) did not induce dimerization of (**1**) but was surprisingly efficient at the *breakup* of aggregates of

other cyanine dyes. Subsequently we hypothesized that Me-β-CD would be the preferred compound to drive efficient cyanine-dye monomer complexation. In the absence of a host, (1) does not form dimers in concentrated aqueous solutions, making it difficult to observe host induced dimer breakup. Hence we chose a different set of cyanine dyes within the benzothiazole and 3,3-dimethyl indolinine series in order to study complexation induced deaggregation.

In an effort to determine the specific structural features of cyanines that induce deaggregation and subsequent inclusion complex formation with Me-β-CD, we have selected eight dyes (Dyes 2 to 9) shown in **Figure 1**. These examples facilitate the spectrophotometric monitoring of dimer breakup or enhanced dimerization with Me-β-CD, if either phenomenon were to occur with a given dye. The primary distinguishing structural features between the dyes are a) the heterocycle *e.g.*, benzothiazole or 1,1' dimethylindolinine, used to construct the cyanine; b) the length of the rigid polymethine chain joining the heterocycles and; c) the length of the flexible quarternized N-alkyl chain.

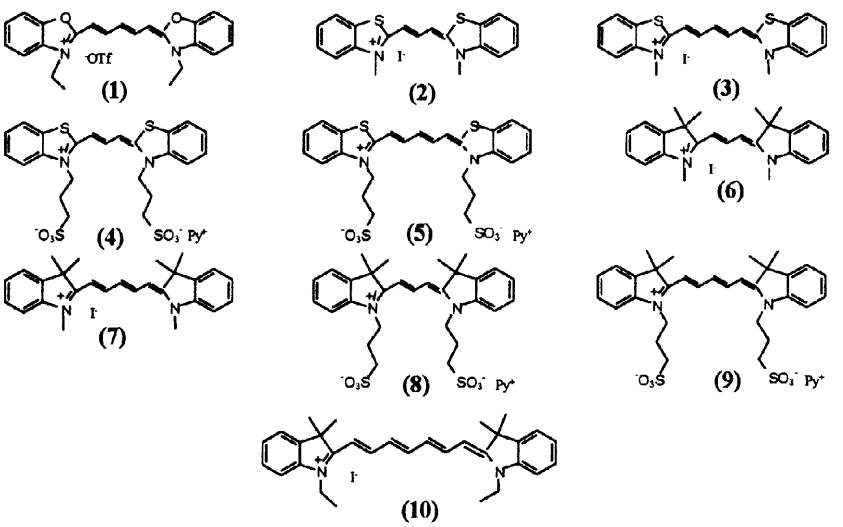

Figure 1

Figure 2 shows the effect of Me-β-CD upon complexation of each of the eight selected dyes. We found that with the exception of (2), the rest of the thiacarbocyanine compounds (3), (4) and (5), undergo a cyclodextrin driven conversion of dimer to the monomer as indicated by the increase in the red shifted absorption band at the expense of the higher energy blue shifted dimer absorption band.[5] In all cases, an equivalent amount of glucose substituted for β-CD did not result in the dimer break up, suggesting that

inclusion is the primary phenomenon leading to dye aggregate break up. Further, in the case of dyes (6), (7), (8), and (9), no noticeable change in the absorption maximum was observed upon additon of Me-β-CD.

Dye Photostability

Photostability of the dyes was determined by plotting loss of absorbance as a function of time. Despite inclusion in the cyclodextrin cavity, photodecomposition of the four thiacarbocyanines was not inhibited

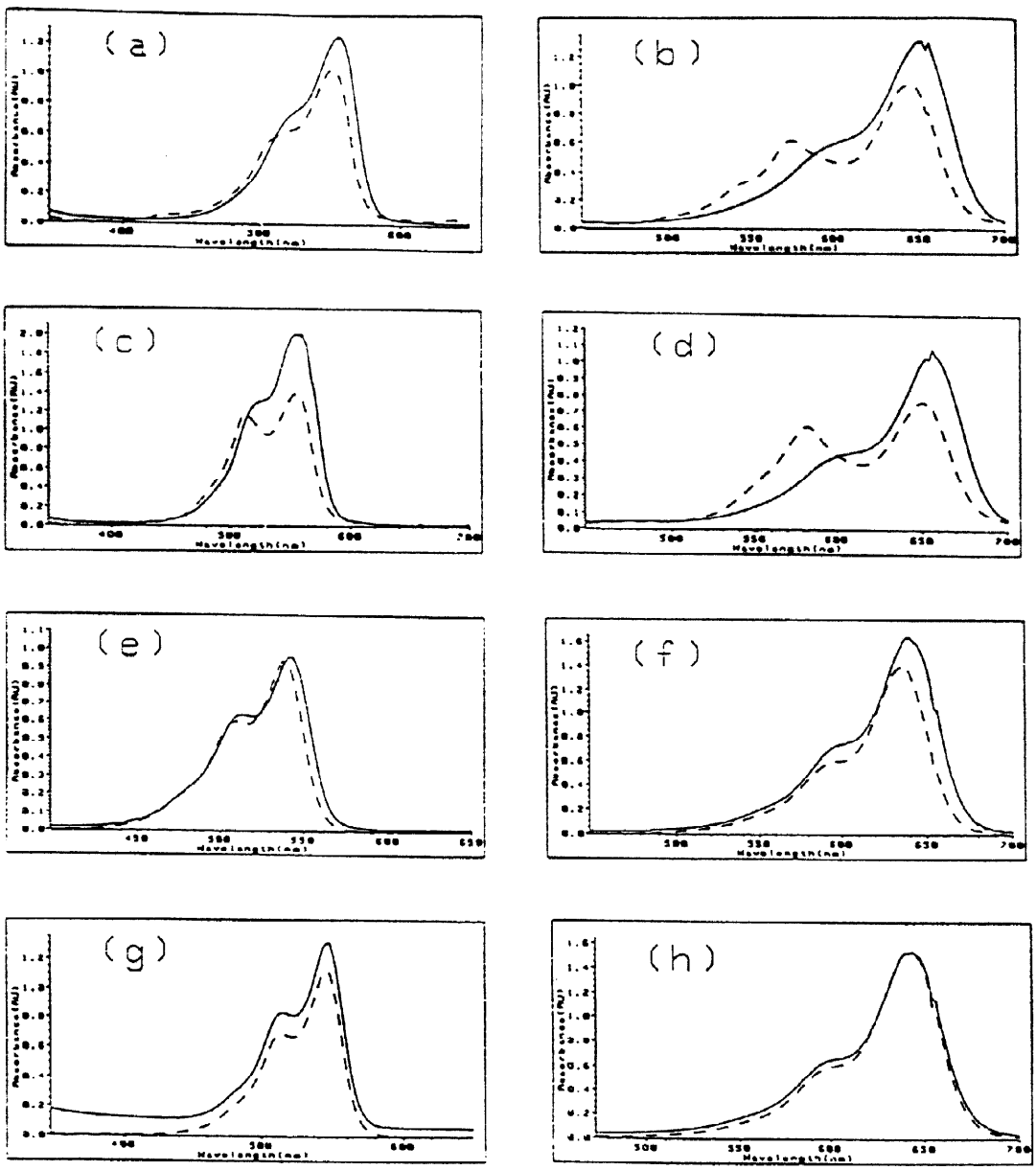

Figure 2. Effect of Me-β-CD on the photostability of cyanine dyes. (a) [Dye **2**] = 0.8 x 10^{-5}M, (b) [Dye **3**] = 1.6 x 10^{-5}M, (c) [Dye **4**] = 1.6 x 10^{-5}M, (d) [Dye **5**] = 0.32x10^{-5}M, (e) [Dye **6**] = 1.0 x 10^{-5}M, (f) [Dye **7**] = 0.8 x 10^{-5}M, (g) [Dye **8**] = 0.8 x 10^{-5}M, (h) [Dye **9**] = 0.6 x 10^{-5}M. A second set of identical aqueous dye solutions containing 9.0 x 10^{-2}M Me-β-CD were prepared to determine the role of cyclodextrins in conveting dye aggregates to monomers. (---: dye solutions; black columns: —: dye solutions containing Me-β-CD)

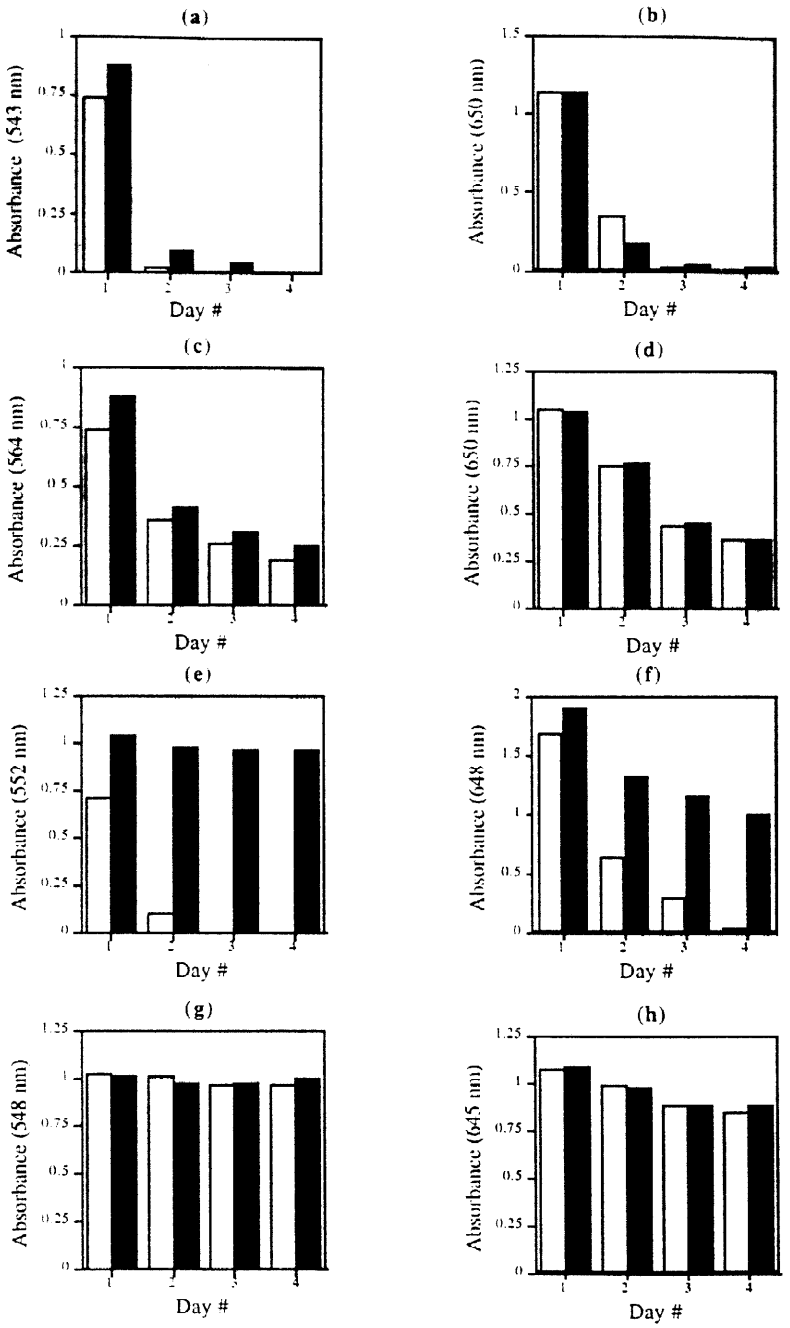

Figure 3. Effect of Me-β-CD on the photostability of cyanine dyes. (a) [Dye **2**] = 0.8 x 10^{-5}M, (b) [Dye **3**] = 1.6 x 10^{-5}M, (c) [Dye **4**] = 1.6 x 10^{-5}M, (d) [Dye **5**] = 0.32x10^{-5}M, (e) [Dye **6**] = 1.0 x 10^{-5}M, (f) [Dye **7**] = 0.8 x 10^{-5}M, (g) [Dye **8**] = 0.8 x 10^{-5}M, (h) [Dye **9**] = 0.6 x 10^{-5}M. A second set of identical aqueous dye solutions containing 9.0 x 10^{-2}M Me-β-CD were prepared to determine the role of cyclodextrins towards controlling the loss of absorbance. (white columns: dye solutions; black columns: dye solutions containing Me-β-CD).

(**Figure 3**). Similar studies with indocarbocyanine dyes produced different results. In the presence of Me-β-CD, dye (**6**) retained starting absorbance for at least five days. In the absence of Me-β-CD the same dye was found to undergo photobleaching as indicated by total loss of absorbance after two days. In the case of (**7**), roughly 60% of the absorbance was retained after four days when complexed with Me-β-CD, but the dye underwent complete photodecomposition after three days without Me-β-CD. There was no effect of complexation on the photostability of (**8**), since the absorbance essentially remained unchanged after five days, both in the presence and absence of Me-β-CD. Lastly, in the case of (**9**), time dependent photodecomposition was observed irrespective of the presence of Me-β-CD. Thus the only conspicuous effect of photostability enhancement via Me-β-CD hosting was observed in the case of (**6**) and (**7**).

DISCUSSION

Cyclodextrins are known to influence the photophysical behaviour of fluorescent dyes via a number of different mechanisms.[14,15,16] Utility of organized assemblies like cyclodextrin complexes in the breakup of molecular aggregates has been previously demonstrated.[17] When provided with appropriate stuctural features, an organic molecule can be included within an open ended molecular cavity such as cavitands and cyclodextrins.[18] This suggests the possibility that a cyclodextrin with predetermined cavity dimensions can influence the photophysical and photochemical properties of cyanine dyes. Our studies were focussed on the assessment of the ability of cyclodextrins to assist in aggregate breakup to reduce fluorescence quenching and enhance photostability of cyanine dyes.

Based on MM2 calculations[19], it has been suggested that incorporation of a cyanine dimer within a γ-cyclodextrin cavity is energetically feasible. It was rationalized by Kasatani *et al* that the destabilization due to CD ring expansion to accomodate the dimeric dye is overcome by the gain in energy due to hydrophobic stabilization by virtue of sandwich dimer (H-dimer) formation. Despite the detailed nature of the study, dominant emphasis was placed solely on the dimer inducing properties of the β- and γ-cyclodextrins. In our laboratories, specific dyes e.g. (**1**) failed to undergo dimerization with Me-β-CD. A narrower cavity[20] at the rim containing the 6-methoxy groups of the methylated cyclodextrin could result in inefficient complexation of the dimeric species. Hence we thought that Me-β-CD would be the preferred compound to drive complexation of the monomeric form of the cyanine dye. The absorption profiles shown in **Figure 2** suggest that cyanines belonging to different heterocyclic series (i.e benzothiazole or 3,3-dimethylindolinine) possess different aggregation properties. Dimerization in the case of dyes (**3**), (**4**) and (**5**) was reduced in the presence of Me-β-CD as indicated by an increase in the red shifted absorbance band. Addition of Me-β-CD to aqueous solutions of indocarbocyanine dyes did not induce a noticeable change in their respective absorption profiles. Presumably, the 3,3'-dimethyl substitution of the indole ring provides sufficient steric bulk to prevent cyclodextrin inclusion while the negatively charged sulfopropyl groups provide a sphere of solvation to minimize aggregation. It is noteworthy that in the case of (**4**) and (**5**), despite the presence of the bulky end groups, inclusion complex formation is apparently facile as indicated by their absorption profiles. These observations suggest that only cyanines with specific structural attributes undergo Me-β-CD complexation driven dimer breakup. The complexation is further manifested in the form of red shifted absorption and enhanced emission intensity.[21]

Although this observation seemingly contradicts past findings[13], close examination reveals that induced dimerization and induced dimer breakup can be selectively achieved with minor variations in the host-guest geometry.

Cyanine dyes that form H-dimers in concentrated aqueous solutions possess enhanced triplet state yields.[22] Singlet oxygen thus produced by energy transfer from an excited state dye molecule has been suggested as a possible causative agent in the photobleaching of cyanine dyes.[23] Inhibition of photobleaching was observed upon complexation of cyanines with cyclodextrins.[24] Matzsuzawa[25] demonstrated a 35% reduction in the quantum yield of photodegradation when a heptamethine cyanine dye was complexed with β-cyclodextrin. Although informative, the study was conducted with a single dye i.e. (**10**) whose inclusion properties as well as susceptibility to photobleaching are no doubt different from other structural homologs of cyanine dyes. Thus we felt the need to conduct a sytematic study to determine the generality of this observation with cyanine dyes containing different atomic and structural parameters. The dyes shown in **Figure 1** were subjected to photobleaching with and without Me-β-CD. We found that cyclodextrin complexation (**Figure 3**) did not inhibit photodecomposition of thiacarbocyanine dyes. Thiacarbocyanines are highly prone to H-dimer formation relative to indocarbocyanine dyes and are rendered monomeric upon complexation with cyclodextrins.[21] According to prior studies, the host included monomeric cyanines are expected to limit singlet oxygen production and subsequent photooxidation.[25] Results (**Figure 3**) to the contrary in the case of thiacarbocyanines can be explained in the following manner. Dyes with low oxidation potential are prone to rapid attack by singlet oxygen.[23] Our observations indicate that despite the protective coat encapsulating the dyes (**2**) to (**5**), low oxidation potential in the case of thiacarbocyanines is an overriding factor in the processes leading to photobleaching. In the case of the indocarbocyanines different results were obtained. The photobleaching of (**6**) was completly inhibited in the presence of Me-β-CD. Dye (**7**) retained only about 60% of the original absorbance after four days when complexed with Me-β-CD. The polymethine chain of of dye (**6**) is shorter in length than that of dye (**7**). As a consequence C(1) and C(1') of (**6**) can be accomodated within the cyclodextrin interior (~ 7.8 °A) and are less available for photooxidation.[25] Dyes (**8**) and (**9**) have a lesser tendency to dimerize and possess a higher oxidation potential. Consequently, the photostability of the two dyes was not seriously affected under ambient conditions and additon of Me-β-CD does not seem to offer any considerable advantage.

Conclusions

The propensity of cyanines toward H-dimer formation in concentrated aqueous solutions depends on the heteroatom substitution and steric factors of a given dye. Although it was shown that γ-CD and in some cases b-CD induced dimer formation of cyanines, we observed that Me-b-CD induced dimer breakup with a concomitant increase in absorbance, as well as the appearance of a red shifted absorbance band, indicating monomer over dimer formation . Modification of specific properties (i.e. deaggregation, enhancement of photostability) by complexation of a given dye is dependent on the structure of the dye as well as the size of the host cavity. β-CD or Me-β-CD induce dimer breakup and selectively encapsulate the monomeric species.

Hence the phenomenon of fluorescence quenching as a result of H-dimer formation can be subdued by inclusion driven dimer breakup. Inclusion also inhibits photodegradation of some cyanine dyes. This phenomenon is not generalized and is highly dependent on the structure of the dye.

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REFERENCES

1. Hamer, F.M.; *The cyanine dyes and related compounds*, Interscience Publishers, 1964.
2. Dexhage, K.H.; *Dye Lasers*, F.R Schafer (Ed), Springer, Berlin, 1977.
3. Busch, W.; *Chromosome Res.*, **1994**, *2*, 15.
4. Herz. A.H.; *Advances in Colloid and Interface Science*, **1977**, *8*, 237.
5. West. W.; Pearce, S.; *J. Phys. Chem.*, **1965**, *69*, 1894.
6. Sato, H.; Kawasaki, M.; Kasatani, K.; Nakashima N.; Yoshihara, K.; *Bull. Chem. Soc. Jpn.*, **1983**,*56*, 3588.
7. Politzer, I.R.; Crago, K.T.; Hampton, T.; Joseph, J.; Boyer, J.H.; Shah, M.; *Chem, Phys. Lett.*, **1989**,*159*, 258.
8. B. Sarkar, B.; Das, U.; Bhattacharya, S.; Bose, S.K.; *Bull. Chem. Soc. Jpn.*, **1995**, *68* , 1807.
9. Herkstroeter, W.G.; Martic, P.A.; Farid, S.; *J. Am. Chem. Soc.*, **1990**, *112*, 3583.
10. Flamingi, L.; *J. Phys. Chem.*, **1993**, *97*, 9566.
11. Giloh, H.; Sedat, J.W.; *Science*, **1982**, *217* , 1252.
12. a) Southwick, P.L., Cairns, J.G., Ernst, L.A., Waggoner, A.S., *Org. Prep. Proced. Int.* **1988**, *20*, 279., b) Hamer, F.H., *J. Chem. Soc.*, 2796, **1927**, c) British Patent 742,112, **1955**, d) Hamer, F.H., Rathbone, R.J., *J. Chem. Soc.*, 595, **1945**, e) For a review on synthesis of symmetric and unsymmetric dyes see: Ficken, G.E., Cyanine Dyes in: *The Chemistry of Synthetic Dyes*; Venkataraman, K., (Ed) Academic press, New York, **1971**. High resolution Mass spectral data: (**2**) Mol. Formula $C_{19}H_{17}N_2S_2$ Calc. Mol wt. 337.0828, found 337.0828, (**3**) Mol. Formula $C_{21}H_{19}N_2S_2$ Calc. Mol. Wt. 363.0985, found 363.0984, (**4**) Mol.Formula $C_{23}H_{27}N_2O_6S_2$ Calc. Mol. Wt. 551.0445, found 551.0444. (**5**) Mol. Formula $C_{25}H_{25}N_2O_6S_4$ Calc. Mol. Wt. 577.610, found 577.601. (**6**) Mol. Formula $C_{25}H_{29}N_2$ Calc. Mol. Wt. 357.2328, found 357.2325. (7) Mol. Formula $C_{27}H_{31}N_2$ Calc. Mol. Wt. 383.2483, found 383.2482.(**8**) Mol. Formula $C_{29}H_{35}N_2O_6S_2$ Calc. Mol. Wt. 571.1951, found 571.1942. (**9**) Mol. Formula $C_{29}H_{37}N_2O_6S_2$ Calc. Mol. Wt. 597.2106, found 597.2099 .
13. Kasatani, K.; Ohashi, M.; Kawasaki, M.; Sato, H.; *Chem. Lett.*, **1987**, 1633; Kasatani, K.; Ohashi, M.; Sato, H.; Carbohydr. Res., **1989**, *192*, 197.
14. Harada, A.; Furue, M.; Nozakura, S.; *Macromolecules*, **1977**, *10* , 676.
15. Bergmark, W.R.; Davis, A.; York.; C, Macintosh, A.; Jones, G.; *J. Phys. Chem.* , **1990**, *94*, 5020.
16. Cho, D.W.; Kim, Y.H.; Kang, S.G.; Yoon, M.; Kim, D.; *J. Phys. Chem.*, **1994**,*98*, 558.
17. a) Dick, D.L.; Rao, T.V.S. ; Sukumaran, D.; Lawrence, D.S.; *J. Am. Chem. Soc.*, **1992**, *114*, 2664., b) Rademacher, J.T.; Czarnik, A.W.; *J. Am. Chem. Soc.*, **1993**, *115*, 3018.
18. Schill, G.; *Catenanes, Rotaxanes and Knots*; Academic Press: New York, 1971.
19. Ohashi, M.; Kasatani, K.; Shinohara, H.;Sato, H.; *J. Am. Chem. Soc.*, **1990**, *112*, 5824.
20. J. Szetli, *Cyclodextrins and their inclusion complexes*, Akademia Kiado, Budapest, 1982.
21. Huff, J.; Cornwell, M.; Venkata,T.; Unpublished results.
22. Pant, D.; Bhagchandani, C.; Pant N.; Verma, S.; *Chem. Phys. Letters.*, **1971**, *9*, 546.
23. Byers, G.W.; Gross, S.; Henrichs, P.M.; *Photochem. Photobiol*, **1972**, *16*, 37.
24. Guether, Y.; Reddington.; *Tet. Lett.*, **1997**, *38*, 6167.
25. Matsuzawa, Y.; Tamura, S.; Matsuzawa, N.; Ata, M.; *J. Chem. Soc. Faraday Trans.*, **1994**, *90 (23)*, 3517.