

# UV/Vis Spectral Study of the Self-aggregation of Pinacyanol Chloride in Ethanol–Water Solutions

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**Abstract** The concentration dependent self-aggregation of 1,1'-diethyl-2,2'-carbocyanine chloride (pinacyanol chloride dye) in 7.5% (v/v) ethanol + 92.5% water solution in the range of  $10^{-6}$ – $10^{-3}$  mol·dm<sup>-3</sup> has been investigated by quantitative UV/vis and derivative spectroscopy. Bands with maxima at 601, 546, 522, and 507 nm could be attributed, respectively, to the monomer, a sandwich-type dimer, a vibronic overtone of the dimer, and a higher aggregate, most probably a stacked trimeric form of the dye. Using the PeakFit program, the overlapping bands were separated and analyzed for all concentrations studied. From the spectral fitting routine, extinction coefficients of 192,000, 156,000, and 282,000 cm<sup>-1</sup>·mol<sup>-1</sup>·dm<sup>3</sup> were determined for the monomer, dimer and trimer respectively.

**Keywords** Pinacyanol · Self-aggregation · UV/vis spectra · Derivative spectroscopy · PeakFit

## 1 Introduction

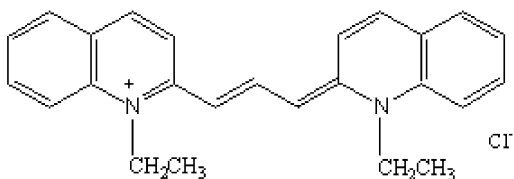
Cyanine dyes are characterized by their intense and sharp absorption bands in the UV/vis region (225–735 nm) with narrow half-band widths of typically about 25 nm [1]. One of the most important applications of cyanine dyes is their use as spectral sensitizers for silver halide micro-crystals in photographic films, since their absorption spectra are easily shifted by chemical substitution and by aggregation [2, 3]. Cyanine dyes have been used frequently to probe biological systems through the measurement of induced circular dichroism (ICD) spectra [4, 5]. They have a natural tendency to self-aggregate in solution when their concentration is increased [6–8]. This aggregation in aqueous solution is affected by strong attractive dispersion reversible forces through the conjugated chain. The electrostatic repulsion between the cationic dye molecules will be reduced due to the relatively high value

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**Fig. 1** Chemical structure of 1,1'-diethyl-2,2'-carbocyanine chloride (pinacyanol chloride)



of the dielectric constant of water [9]. Self-aggregation of cyanine dyes can also occur in mixtures of organic solvents with water [10], depending on the temperature and the nature of the solvent.

The absorption spectra of aggregated molecules are distinctly different from those of the monomeric species. From the spectral shifts, various aggregation types have been proposed. H-type aggregates are formed when the dye molecules arrange themselves face-to-face in near vertical stacks with blue shifts of the absorption maxima, which depend quantitatively on the number of monomers in the aggregate [7]. J-aggregates are formed when the dye molecules arrange into slanted stacks with red shifts of the absorbance [11].

In the present study, we have investigated the self-aggregation of 1,1'-diethyl-2,2'-carbocyanine chloride (pinacyanol chloride) in ethanol–water solutions by quantitative UV/vis spectroscopy. This symmetric cationic polymethine dye (Fig. 1) is widely used as a saturable absorber, mode-locker, or sensitizers in photography [8, 12]. The normal and derivative (mainly fourth) absorption spectra were used to determine the maxima of the strongly overlapping bands, which belong to monomer, dimer, and other aggregates forms of the dye, followed by an analysis of these components using a non-linear peak fitting program, which yielded the positions and intensities of the resolved spectra.

## 2 Materials and Methods

### 2.1 Materials

1,1'-diethyl-2,2'-carbocyanine chloride (pinacyanol chloride) was obtained from Sigma and spectrophotometric grade ethanol from Merck. Triply distilled water was used throughout the study.

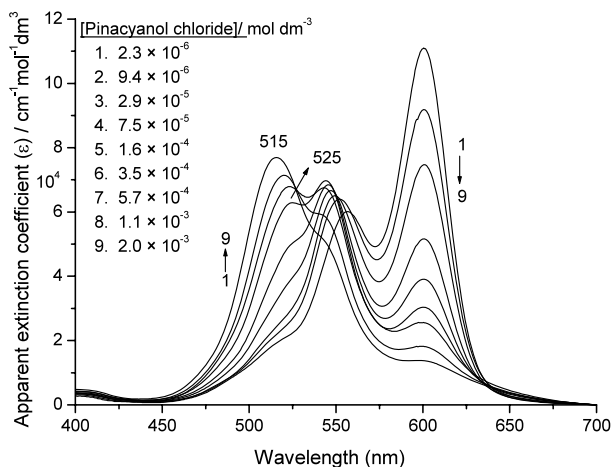
### 2.2 Experimental Methods

The solutions were prepared by using micropipettes of different capacities (100, 50, and 10  $\mu\text{L}$ ) to a fixed volume (4.00 mL) using stoppered, rolled rim, flasks, to maintain the same conditions and precision for all samples. Quartz cells with optical path lengths ranging from 0.01 to 2.00 cm were used as appropriate. Dye solutions were used on the day of preparation. UV/vis spectra were obtained using a Perkin-Elmer Lambda 5 spectrophotometer connected to a personal computer for data collection in ASCII-file format.

## 3 Results and Discussion

Figure 2 shows the UV/vis spectra of pinacyanol chloride solutions (ranging from  $2.3 \times 10^{-6}$  to  $2.0 \times 10^{-3}$  mol·dm $^{-3}$ ) with 7.5% (v/v) ethanol + 92.5% water at 25 °C. Ethanol was added to increase the solubility of the dye, and thus increase the concentration range that

**Fig. 2** Concentration dependent UV/vis spectra of pinacyanol chloride in water at room temperature (7.5% (v/v) ethanol has been added to increase solubility). The optical path lengths is 2.00 cm for solutions #1 and #2, 0.50 cm for solution #3, 0.20 cm for solution #4, 0.10 cm for solution #5, 0.05 cm for solution #6, and 0.01 cm for solutions #7, #8, and #9



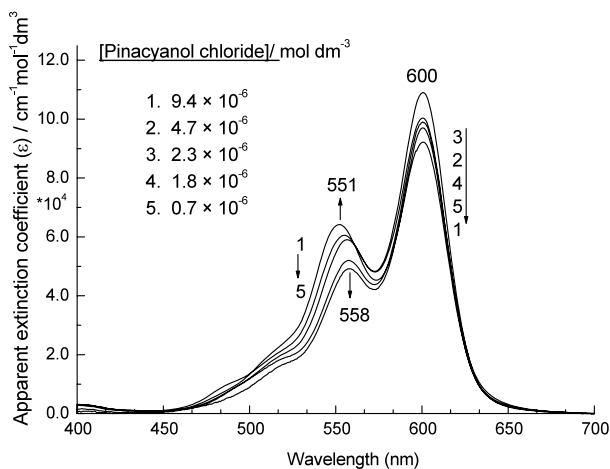
could be studied. The amplitudes are given in terms of the apparent extinction coefficient ( $\epsilon$ ), which is obtained by dividing the measured absorbance of the dye solution by its total molar concentration (as monomer form) and the path length of the cell.

The spectrum with the lowest dye concentration shows two main absorption bands, one centered at 600 nm ( $16,666 \text{ cm}^{-1}$ ) and the other at 555 nm ( $18,018 \text{ cm}^{-1}$ ). In addition, there is a weak shoulder around 520 nm ( $19,230 \text{ cm}^{-1}$ ). At this concentration the absorption band at 600 nm is the most intense one, which corresponds to the  $0 \rightarrow 0$  vibronic transition between the electronic molecular ground state and the first excited one in the pinacyanol dye molecule. Since at this low concentration there is no indication of aggregated species being present, the other two absorptions must correspond to monomer absorptions as well. We tentatively assign them to the  $0 \rightarrow 1$  and  $0 \rightarrow 2$  vibrational excitation of the ground to the first excited state. This is in line with the almost constant energy shifts of  $1,352$  and  $1,212 \text{ cm}^{-1}$ . Similar separations of excited state vibrational mode energies have been reported for other cyanine dyes [7].

Increasing the dye concentration causes molecules to aggregate, which leads to changes in the absorption spectrum and deviations from the Beer-Lambert law. As a result, the extinction coefficient values become a function of the pinacyanol chloride concentration. There are three main effects, which can be observed as the pinacyanol chloride concentration increases: 1) The monomer absorbance at 600 nm continuously decreases. 2) The band at 555 nm is blue-shifted to 545 nm. It reaches its maximum intensity at a concentration of  $3.5 \times 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$ . 3) The shoulder around 520 nm increases in intensity throughout the concentration range. It is red-shifted first into a band with maximum at 525 nm at  $3.5 \times 10^{-4} \text{ mol} \cdot \text{dm}^{-3}$ , and then blue-shifted to 515 nm when a concentration of  $2.0 \times 10^{-3} \text{ mol} \cdot \text{dm}^{-3}$  is reached. This is the highest dye concentration that could be obtained. The path length at this concentration was 0.01 cm, and there were difficulties caused by dye precipitation at concentration higher than this.

For a quantitative analysis of these concentration dependent effects, one has to take into account the fact that the monomer overtone band at 555 nm overlaps strongly with the dimer band at 545 nm. This means that the 545 nm absorbance is not due to the dimer alone. In contrast, the long wavelength absorbance at 600 nm is a reasonable measure of the monomer concentration of pinacyanol chloride. Figure 3 presents spectra of pinacyanol solutions with concentrations as low as  $0.7 \times 10^{-6} \text{ mol} \cdot \text{dm}^{-3}$  where most of the molecules can be assumed to be present as monomers. Unfortunately, absorption of dye molecules on the cell walls

**Fig. 3** The visible absorption spectra of pinacyanol chloride solution with 7.5% (v/v) ethanol + 92.5% water added at 25 °C, showing mainly the monomer vibronic bands. The optical path length is 1.00 cm for solution #1, and 2.00 cm for others

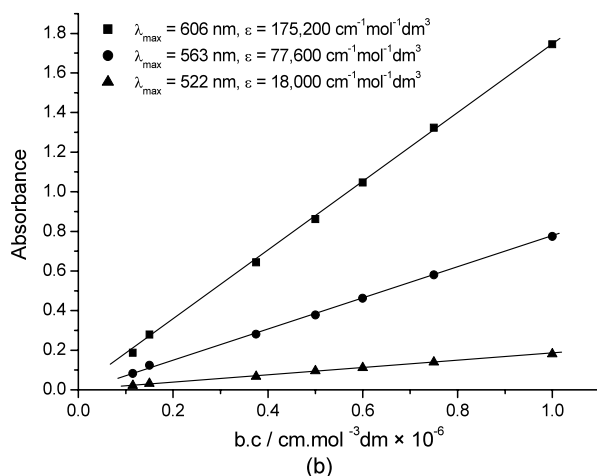
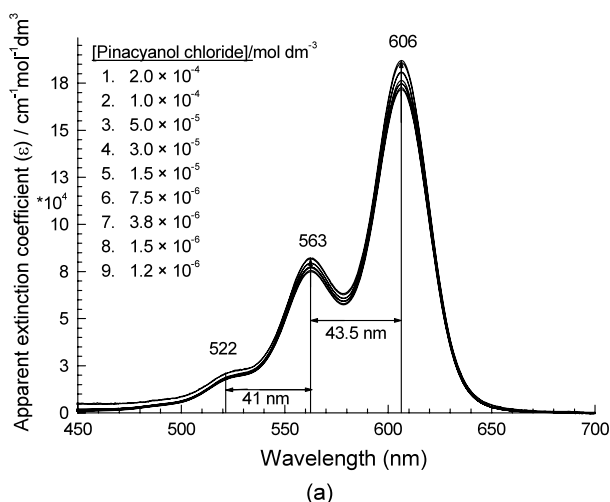


becomes a significant perturbation at very low dye concentrations leading to a decrease in the absorbancies at 600 nm and at 555 nm as can be seen in Fig. 3. However, the band remaining constant at 600 nm and the blue shift of the second band from 558 to 551 nm indicate, even at these low concentrations, an equilibrium between monomeric and dimeric species of pinacyanol dye.

For comparison, Fig. 4 shows the concentration dependent UV/vis spectra of pinacyanol chloride in absolute ethanol, a solvent which is known to impede dye aggregation. There are two absorption maxima at 606 ( $16,500\text{ cm}^{-1}$ ) and 563 nm ( $17,777\text{ cm}^{-1}$ ) in addition to a shoulder around 520 nm. These features are independent of the concentration in the range from  $10^{-6}$  to  $10^{-4}\text{ mol}\cdot\text{dm}^{-3}$  (Fig. 4a) indicating that, under these conditions, no dimers or higher aggregates are formed. This is supported by the Beer-Lambert law plot of the two absorbance maxima at 606 and 563 nm, Fig. 4b. The absence of any deviation in these plots proves that there is only one component of pinacyanol chloride in ethanol in this range of concentrations at 25 °C.

From the linear plots displayed in Fig. 4b, values of the extinction coefficients of monomeric pinacyanol chloride in ethanol of  $175,200$ ,  $77,600$ , and  $18,000\text{ cm}^{-1}\cdot\text{mol}^{-1}\cdot\text{dm}^3$  at 606, 563, and 522 nm, respectively, can be derived. The energy difference between the main peak at 606 nm and the second vibronic peak at 563 nm is  $1,290\text{ cm}^{-1}$ , and between the second vibronic peak and the vibronic shoulder around 522 nm is  $1,390\text{ cm}^{-1}$ . The ratio of the absorbance intensities between 563 nm and 606 nm is 0.44, and between 522 nm and 606 nm it is 0.10. We assume that these ratios are constant, or very nearly so, in other solvents, and we have used them in the analysis of the UV/vis spectra of pinacyanol chloride in ethanol–water solutions.

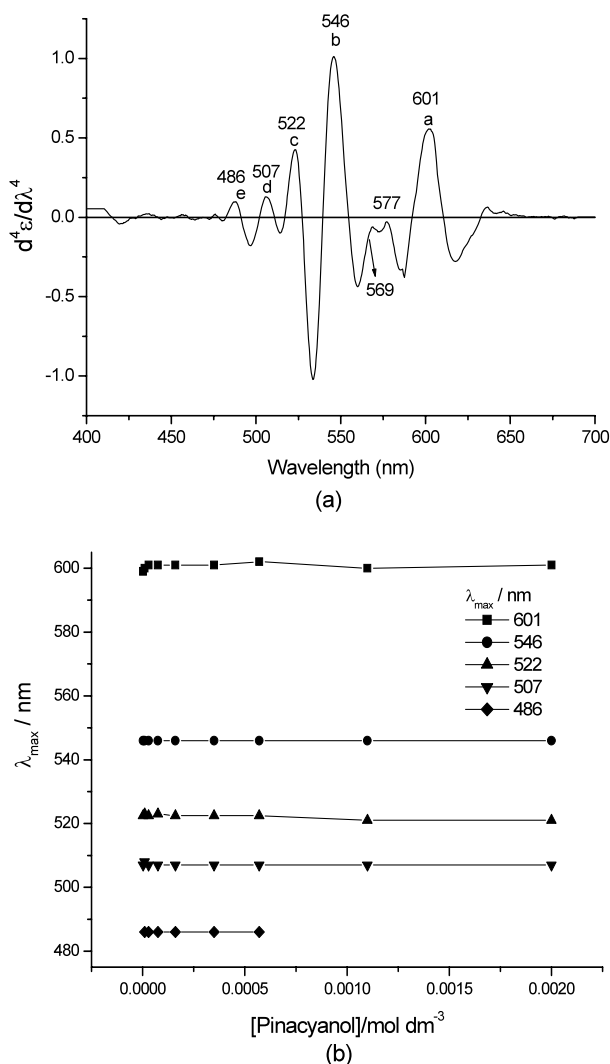
From Fig. 2, it is difficult to determine the exact wavelengths of the different maxima and shoulders directly. Derivative spectroscopy can facilitate identification of peak positions and quantitative analysis of the complex spectrum. The term derivative spectroscopy refers to a spectral measurement technique in which the slope of the spectrum (the rate change of absorbance  $A$  with wavelength  $\lambda$ ) is measured as a function of the wavelength [13–15]. Figure 5a presents, as an example, the fourth-derivative spectrum of pinacyanol chloride at  $7.5 \times 10^{-5}\text{ mol}\cdot\text{dm}^{-3}$  (spectrum 4 in Fig. 2). In Fig. 5a, the fourth-derivative spectra denoted by a, b, c, d, and e indicate absorptions of the monomer, dimer, and other aggregates, respectively, which can be correlated with the derivative spectra. Repeating the same procedure for



**Fig. 4** (a) The visible absorption spectra of pinacyanol chloride in pure ethanol at room temperature with different concentrations showing the monomer bands of absorptions. (b) Beer-Lambert law plot of pinacyanol chloride solutions in pure ethanol at 606, 563 and 522 nm. The optical path length is 0.01 cm for solution #1, 0.05 cm for solution #2, 0.10 cm for solutions #3–#5, 0.50 cm for solutions #6 and #7, and 1.00 cm for solutions #8 and #9

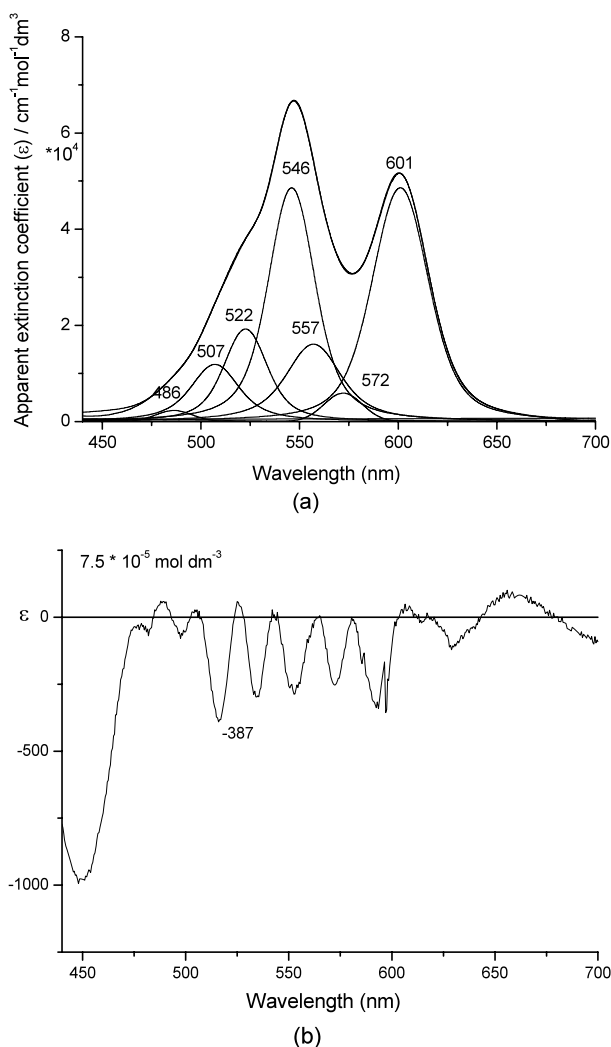
the other eight concentrations of pinacyanol chloride solutions shown in Fig. 2, we obtain the maximum wavelengths for each component of pinacyanol chloride at each concentration. Figure 5b shows the maximum wavelengths for monomer, dimer, and the other forms of aggregates, which are taken from the fourth-derivative spectra by identifying the position of the peak maxima. These wavelengths are independent of the concentration at which the measurements were performed.

PeakFit is non-linear peak-fitting program from Systat software Inc. in which the analyzed data sets are directly imported from the raw ASCII data that were measured directly by the spectrophotometer. The Voigt function was chosen for the analytical computation and the line shape arising as a result of the convolution of the Lorentzian and Gaussian



**Fig. 5** (a) The fourth-derivative spectrum of  $7.5 \times 10^{-5}$  mol·dm<sup>-3</sup> pinacyanol chloride solution with 7.5% (v/v) ethanol + 92.5% water at 25 °C in a 0.10 cm cell. (b) The wavelengths of the different absorption maxima of pinacyanol chloride at different concentrations obtained from the fourth-derivative spectra

components within a spectroscopic peak. Figure 6a shows a sample result of the resolved absorption bands for the  $7.5 \times 10^{-5}$  mol·dm<sup>-3</sup> pinacyanol chloride spectrum. They are, starting with the longest wavelength (from the right): the monomer band at 601 nm, the first vibronic overtone at 557 nm, the dimer at 546 nm, the second vibronic overtone (or maybe another type of aggregation) at 522 nm, and other aggregates at 507 and 486 nm. The  $\lambda_{\max}$  values obtained from the PeakFit program are in very good agreement with the values obtained from the fourth-derivative spectra (Fig. 5b). The graph of the residuals (Fig. 6b), which depicts the difference between the experimental absorption spectrum and that calcu-



**Fig. 6** (a) The absorption spectrum of the  $7.5 \times 10^{-5} \text{ mol}\cdot\text{dm}^{-3}$  pinacyanol chloride solution resolved into its components with the PeakFit program. (b) The graph of the residuals that belongs to (a)

lated using the PeakFit program on the basis of the different components, shows absolute values that are statistically highly acceptable, with deviations of less than 1%.

Table 1 summarizes the information obtained from the analysis of the spectra shown in Fig. 2 using the PeakFit program. In the table, the absorbancies are calculated from extinction coefficients that were taken directly from the PeakFit program. Using the data of Table 1, we can form nine different equations with three unknown values of the extinction coefficients for the pinacyanol chloride bands, with maximum wavelengths at 601, 546, and 507 nm, and which may be attributed to the monomer, dimer, and a higher aggregate, respectively. We assume the bands at 546, 522, and 486 nm are vibronic bands of the dimer rather than bands from different forms of dimer. In the calculations, one wavelength relating to the dimer is used, this is the main band, with the greatest intensity (546 nm) and the

**Table 1** Absorbance values of the resolved peaks of all spectra in Fig. 2 that were obtained from using the PeakFit program

Soln No.	Total concentration/ mol·dm <sup>-3</sup>	Path length/ cm	Absorbance at 601 nm	Absorbance at 557 nm	Absorbance at 546 nm	Absorbance at 522 nm	Absorbance at 507 nm	Absorbance at 486 nm
1	$2.3 \times 10^{-6}$	2.00	0.499	0.148	0.116	0.049	0.040	0.010
2	$9.7 \times 10^{-6}$	2.00	1.771	0.566	0.634	0.266	0.179	0.083
3	$2.9 \times 10^{-5}$	0.50	1.045	0.347	0.594	0.211	0.145	0.030
4	$7.5 \times 10^{-5}$	0.20	0.729	0.241	0.729	0.289	0.178	0.034
5	$1.6 \times 10^{-4}$	0.10	0.589	0.179	0.851	0.437	0.201	0.055
6	$3.5 \times 10^{-4}$	0.05	0.464	0.137	0.914	0.688	0.302	0.093
7	$5.7 \times 10^{-4}$	0.01	0.122	0.036	0.289	0.244	0.127	0.041
8	$1.1 \times 10^{-3}$	0.01	0.166	0.047	0.485	0.408	0.362	0.042
9	$2.0 \times 10^{-3}$	0.01	0.231	0.064	0.729	0.655	0.993	0.096

other bands, at 522 and 486 nm were not included. The total concentration ( $c_t$ ) of the dye (expressed as monomer concentration) for each of the nine different solutions is equal to the sum of concentrations of all components:

$$c_t = c_m + 2c_d + nc_h \quad (1)$$

where  $c_m$ ,  $c_d$ , and  $c_h$ , are the concentrations of the monomer, dimer and the higher aggregate respectively. The nine different equations (that can be formed by using the data in Table 1) have the general form:

$$bc_t = A_m/\varepsilon_m + 2A_d/\varepsilon_d + nA_h/\varepsilon_h \quad (2)$$

where,  $b$  is the path length of the cell,  $A$  is the absorbance and  $\varepsilon$  is the extinction coefficient. As an example, the equation of the solution 1 can be written according to Eq. 2 as:

$$4.60 \times 10^{-6} = 0.499/\varepsilon_m + 0.232/\varepsilon_d + 0.040n/\varepsilon_h \quad (3)$$

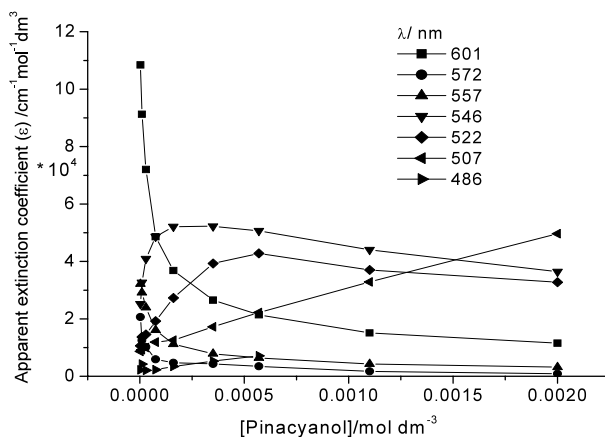
where  $n = 3$  if the band at 507 nm belongs to the trimer form, or  $n = 4$  if the band at 507 nm belongs to tetramer form. Using a 3-unknown calculation for all nine equations, the calculated values of the three unknown extinction coefficients are  $\varepsilon_m = 192,000 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$ ,  $\varepsilon_d = 156,000 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$ , and  $\varepsilon_h = 282,000 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$  (for trimers with  $n = 3$ ) or  $\varepsilon_h = 376,500 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$  (for tetramers with  $n = 4$ ).

If the bands at 546, 522, and 486 nm belong to different sorts of dimer, five unknowns should be included in the calculations. Calculations using five unknowns gave no reasonable results and so we are of the opinion that the bands at 546, 522 and 486 nm belong to the same sort of dimer, and we have removed the bands at 522 and 486 nm from our calculations.

The band at 522 nm can be interpreted in two different ways; either this band corresponds to a vibrational overtone of the dimer band (546 nm), or it represents a dimer of a different geometry and therefore different absorbance. The energy splitting of the two bands at 546 and 522 nm of  $842 \text{ cm}^{-1}$  is in the same range, but significantly smaller than for the monomer. However, the presence of a different dimer cannot be ruled out. The 507 nm band shows



**Fig. 7** The amplitudes for each component of pinacyanol chloride at different concentrations obtained by using the PeakFit program



a completely different behavior than the 522 nm band, since its height increases almost linearly above a certain concentration. Especially puzzling is the fact that even at the highest concentrations the band does not show any levelling off, which one would expect if there were significant dissociation and aggregation into an even larger aggregate. In the absence of more information we assign this peak to a trimer or higher aggregate.

From the absorbances and the cell constants given in Table 1 and the calculated values of extinction coefficients, the total dye concentration can be calculated according to Eq. 2 with  $n = 3$ . The calculated total dye concentrations ( $c_t$ ) for the nine different solutions are in good agreement with the total dye concentration used to prepare the samples, with an error of less than 1%. From the slope of the absorbance of the monomer form in absolute ethanol at 606 nm against concentration (Fig. 4b), an extinction coefficient of  $175,200 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$  is obtained. The difference between this value and the value found with the PeakFit program ( $190,000 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$ ) may reflect a solvent effect and the different method that was used. The extinction coefficient of the pinacyanol chloride dimer, that is contained within a  $\gamma$ -cyclodextrin cavity, was estimated from the visible spectra [15], with a value of  $142,000 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$ , which is in good agreement with the value calculated by using the PeakFit program ( $153,400 \text{ cm}^{-1} \cdot \text{mol}^{-1} \cdot \text{dm}^3$ ).

A blue shift in the maximum wavelength of the visible absorption band of a dye molecule indicates near vertical stack aggregation of the dye molecules and depends on the number of monomers in the aggregate. In addition to the dimer, which absorbs at 546 nm and considering the calculated extinction coefficients, we attribute the band at 507 nm to the trimeric state of the dye. Formation of higher aggregates is possible in equilibrium with or at the cost of dimer and trimer aggregates. Experimentally, such higher aggregates are difficult to detect due to their very low concentrations and the overlap of their bands with the intense bands of the dimer and trimer aggregates. The aggregate absorbing at 486 nm behaves like another dimer form (Fig. 7); increasing the total dye concentration reduces the intensity of this band, which eventually disappears completely. A new band with relatively low intensity, which appears at 476 nm, may be attributed to a higher aggregated form than the trimer.

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## References

1. Berova, N., Nakanishi, K.: Exciton chirality method. In: Berova, N., Nakanishi, K., Woody, R. (eds.) *Circular Dichroism—Principles and Applications*, pp. 337–382. Wiley-VCH, New York (2000)
2. Causgrove, T.P., Yang, S., Struve, W.S.: Electronic excitation transport in core antennae of enriched photosystem I particles from spinach chloroplasts. *J. Phys. Chem.* **92**, 6121–6124 (1988)
3. Lanzafame, J.M., Muentner, A.A., Brumbaugh, D.V.: The effect of J-aggregate size on photoinduced charge transfer processes for dye-sensitized silver halides. *Chem. Phys.* **210**, 79–89 (1996)
4. Buss, V., Reichardt, C.: Chiral dimers of a chiral pentamethine cyanine dye and of an achiral pentamethine cyanine dye in  $\gamma$ -cyclodextrin as a chiral host. *J. Chem. Soc. Chem. Commun.* 1636–1638 (1992)
5. Khouri, S.J., Knierim, R., Buss, V.: Induced circular dichroism of the interaction between pinacyanol and algal alginates. *Carbohydr. Res.* **344**, 1729–1733 (2009)
6. Scheibe, G.: Reversible polymerisation als ursache neuartiger. Absorptionsbanden von farbstoffen. *Kolloid Z.* **82**, 1–14 (1938)
7. West, W., Pearce, S.: The dimeric state of cyanine dyes. *J. Phys. Chem.* **69**, 1894–1903 (1965)
8. Min, H., Park, J., Yu, J., Kim, D.: The spectroscopic studies on the aggregation behavior of cyanine dyes. *Bull. Korean Chem. Soc.* **19**, 650–654 (1998)
9. Sabaté, R., Estelrich, J.: Determination of micellar microenvironment of pinacyanol by visible spectroscopy. *J. Phys. Chem. B* **107**, 4137–4142 (2003)
10. Arbeloa, F.L., Ojeda, P.R., Arbeloa, I.L.: Dimerization and trimerization of rhodamine 6G in aqueous solution: effect on fluorescence quantum yield. *J. Chem. Soc., Faraday Trans. 2* **84**, 1903–1912 (1988)
11. Akins, D.L., Macklin, J.W.: Dependence of Raman scattering by aggregated 2,2-cyanine on pH and excitation wavelength. *J. Phys. Chem.* **93**, 5999–6007 (1989)
12. Barazzouk, S., Lee, H., Hotchandani, S., Kamat, P.V.: Excited pinacyanol H-aggregates and their interaction with SiO<sub>2</sub> and SnO<sub>2</sub> nanoparticles. *J. Phys. Chem. B* **104**, 3616–3623 (2000)
13. Thomas, M.: *Ultraviolet and visible spectroscopy*, 2nd edn. Wiley, Chichester (1996)
14. Antonov, L., Nedeltchera, D.: Resolution of overlapping UV-Vis absorption bands and quantitative analysis. *Chem. Soc. Rev.* **29**, 217–227 (2000)
15. Khouri, S.J., Richter, D., Buss, V.: Circular dichroism and theoretical calculations of pinacyanol dimer inclusion in  $\gamma$ -cyclodextrin. *J. Incl. Phenom. Macrocycl. Chem.* (2009). doi:[10.1007/s10847-009-9579-9](https://doi.org/10.1007/s10847-009-9579-9)