A Spectroscopy Study of the Interaction of Pinacyanol with *n*-dodecyltrimethylammonium Bromide Micelles

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Received March 26, 2001. In Final Form: June 26, 2001

The interaction of pinacyanol, a cyanine dye (PIN), with *n*-dodecyltrimethylammonium bromide (DTAB) at micellar concentration range was studied by visible spectrophotometry. As PIN is present in aggregate and nonaggregate forms, we aimed to determine the dimerization constant ($K_D = 35{,}000\ M^{-1}$) by nonlinear regression fitting of the experimental spectra and then by the resolution of such spectra in terms of pure monomer and dimer states. Each state was deconvoluted into three Gaussian bands. These functions fitted the spectral data. Interaction of PIN with micellar DTAB produced a bathochromic shift of all the spectral bands and increased the most red-shifted band. Spectral data showed that the dye is bound into the micelle in the monomeric form. On the other hand, micellization reduced the dimerization process. The binding of the dye to micelles is defined by an association constant ($K_A = 2160\ M^{-1}$). In such binding, one is the maximum number of PIN molecules that each micelle can accommodate. The association constant allowed us to calculate the fraction of micellized PIN, from which we deduced the spectrum of the dye into the micelle. This spectrum was consistent with that of the monomer in a medium with a lower dielectric constant.

Introduction

Cyanine dyes have frequently been used as optical probes in the study of membranes, surfactants, and micellar systems. In particular, methylene blue and merocyanine-540 are widely applied.[1−8]

Pinacyanol (1,1'-diethyl-2,2'-carbocyanine) chloride (PIN) is a cationic dye that belongs to the class of conjugated cyanine dyes. The amphipathic nature of these dyes confers solubility in a wide range of solvents, including water and chloroform. It can form aggregates. A strong dispersion force associated with the high polarizability of the chromophoric chain favors the aggregation of cyanine dyes in aqueous solution. The high dielectric constant of water facilitates the aggregation process by reducing the electrostatic repulsion between similarly charged dye molecules. Aggregation also occurs in mixed solvents and heterogeneous media, that is, micelles.[9]

PIN has been used to determine critical micelle concentrations[10] and to study bacterial polysaccharides[11,12] and the photoisomerization of polymethine dyes, since it forms short-lived photoisomers after the transition $S_0 \rightarrow S_1$ excitation.[13] As a redox indicator, it is used to monitor peroxide activation.[14] In health sciences, it has been observed that PIN causes respiratory immunogenicity.[15]

On the other hand, the λ_{max} of its visible spectrum correlates well with the dielectric constant of the solvent,[16] and it can thus be used as a solvatochromic dye. A reduction in the polarity of the medium produces a bathochromic shift of peaks of maximal absorption, since the large electric dipole moment of the emitting electronic state involves charge transfer and is stabilized in more polar solvents.

The visible absorption spectrum of PIN consists of three overlapping spectral components, with the most intense band red-shifted relative to the others.[17] In water, the maxima of such spectral components can be located at 600, 550, and ≈ 520 nm. The first is usually interpreted as the vibrationless electronic $S_0 \rightarrow S_1$ transition and the other two as the same electronic transition under vibration cooperation. The existence of two multiband spectra corresponding to the monomer and dimer forms of the dye, respectively, has been supported in pinacyanol or close derivatives,[9,18,19] as well as in other cyanine dyes.[2,20]

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10.1021/la010463y CCC: $20.00
Published on Web 09/14/2001

At increasing dye concentrations, the spectrum varies: the peak at 600 nm diminishes, whereas that at 550 nm is enhanced. When the dye is adsorbed on colloidal surfaces, these bands decrease while a new absorption band appears at 495 nm.[9] This is caused by the presence of aggregate forms of PIN (dimers, trimers, and higher aggregates). As the dimer is always in equilibrium with the monomer, the observed absorption spectrum, even at low dye concentrations, is a superposition of the spectra of the monomer and the dimer.

Surfactants (above or below their critical micellar concentrations) affect the electronic absorption spectra of many dyes.[21−23] Hence, spectroscopic techniques can be used to determine certain physicochemical properties of micelles and vesicles.[24] The location of PIN in micelles of some trimethylammonium bromide surfactants has been studied spectroscopically and by acid−base equilibrium elsewhere, suggesting a cation-π interaction between the uncharged ring system of the dye and the cationic headgroups of the surfactants.[25]

Here, the interaction of PIN with *n*-dodecyl-trimethylammonium bromide (DTAB), a cationic surfactant, at micellar concentrations was studied by absorption spectroscopy. Curve-fitting techniques were applied to obtain the dimerization constant and to decompose and analyze the absorption spectrum in terms of component bands. This approach permits a much more precise and detailed interpretation of the dye spectrum, from which the PIN/DTAB association constants are deduced.

Experimental Procedures

Materials. PIN was from Sigma (St. Louis, MO) and *n*-dodecyltrimethylammonium bromide $C_{15}H_{34}NBr$ (DTAB) was from Fluka (Buchs, Switzerland). The purity of DTAB was confirmed by the agreement between the cmc value obtained from conductivity measurements and literature values.[26] Organic solvents, when possible of spectroscopic grade, were from Merck (Darmstadt, Germany). Solutions were prepared in double-distilled water purified through a Milli-Q system (Millipore, USA).

Solution Preparation. Stock solutions of PIN were prepared in $CHCl_3$. When necessary, the required amount was placed in a round-bottomed flask and evaporated at reduced pressure. To remove $CHCl_3$ traces, the residue was freeze-dried overnight and the appropriate solution was prepared. All PIN preparations were kept in the dark and wrapped in aluminum foil to avoid photodecomposition during storage. To minimize dye adsorption, glassware and cuvettes were silanated with 1% (v:v) dichloromethylsilane/toluene solution and then rinsed with methanol.

Spectroscopic Measurements. Visible absorption spectra were recorded with a Shimadzu UV-2401 PC UV−visible spectrophotometer (Shimadzu, Japan) using a matched pair of glass cuvettes of 1 cm optical length placed in a thermostated cell holder at 25 ± 0.1 °C. The spectra were fitted to three overlapping Gaussian curves with the help of a Gaussian curve fitting program that provided the amplitude, center, bandwidth at half of the maximum amplitude and area of each Gaussian function.

Determination of the Dimerization Constant. At low concentrations, PIN is primarily in the form of monomers and dimers. If we neglect the effect of higher aggregates, the equilibrium between monomer and dimer ($2\,M \leftrightarrow D$) is described by the dimerization constant K_D, which is given by the ratio between the molar concentrations of dimers, C_D, and monomers, C_M, at equilibrium:

$$K_D = C_D/C_M^2 \tag{1}$$

Thus, we need known values of C_D and C_M, which, in turn, can be determined from the molar absorptivity obtained from the spectral bands of monomeric and dimeric species.

The total absorbance of a PIN solution per unity of optical length at a given wavelength ($A(\lambda)$) is

$$A(\lambda) = \epsilon_M(\lambda)\cdot C_M + \epsilon_D(\lambda)\cdot C_D \tag{2}$$

where ϵ_M and ϵ_D represent the molar absorption coefficients of monomeric and dimeric species, respectively, of any band at a wavelength λ.

The monomer and dimer concentrations can be calculated from eq 1 considering the mass balance of PIN in the volume dispersion

$$C = C_M + 2\,C_D \tag{3}$$

where C is the total analytical concentration of PIN.

Insertion of eq 1 and 3 into eq 2 affords the following expression:

$$A(\lambda) = \epsilon_D(\lambda)\left(\frac{C}{2} - \frac{-1 \pm \sqrt{1 + 8\cdot K_D\cdot C}}{8\cdot K_D}\right) + \epsilon_M(\lambda)\left(\frac{-1 \pm \sqrt{1+8\cdot K_D\cdot C}}{4\cdot K_D}\right) \tag{4}$$

By plotting the measured absorbances as a function of dye concentration at any wavelength, the molar absorptivity of monomers, $\epsilon_M(\lambda)$, and dimers, $\epsilon_D(\lambda)$, as well as the dimerization constant, K_D, were calculated using a nonlinear least-squares fitting routine (Microsoft Excel Solver).

Determination of the Micelle Association Constant. The micelle association constant, K_A, was calculated from[27]

$$Area_{obs} = Area_w + \frac{Area_{mic} - Area_w}{\frac{1}{K_A(M)^n} + 1} \tag{5}$$

where $Area_{obs}$ is the area under the curve of the overlapping Gaussian bands of the experimental absorption spectrum; $Area_w$ and $Area_{mic}$ are the area under the curve of spectra of PIN in the absence of surfactant and when the maximal amount of PIN is into the DTAB micelles, respectively, n is the number of monomers of the dye associated with the micelle, and M is the concentration of micelles obtained from

$$M = \frac{S - cmc}{N} \tag{6}$$

where S is the surfactant concentration, cmc is the critical micellar concentration (0.0155 M for DTAB in water at 25 °C), and N is the number of aggregation, which depends on the surfactant concentration.[28]

Results and Discussion

Effects of Dye Concentration on the Spectra in Water. Figure 1 shows the visible absorption spectrum of PIN at several concentrations ranging from $2.5\cdot10^{-6}$ to 10^{-5} M. To clarify the spectral tendency, the spectra are given in terms of apparent molar absorptivity rather than absorbance. Changes in molar absorptivity and the presence of a well-defined isosbestic point at 562.2 ± 0.2

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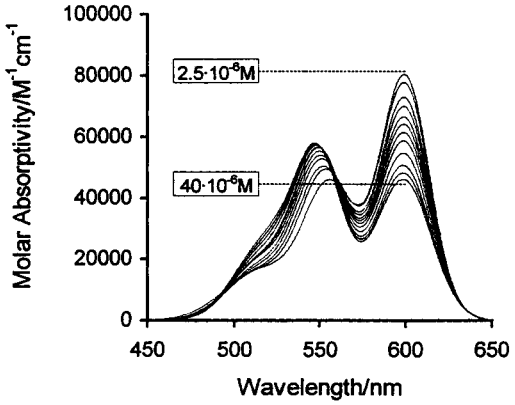

Figure 1. Concentration change in the visible absorption spectra of PIN in water at 25 °C.

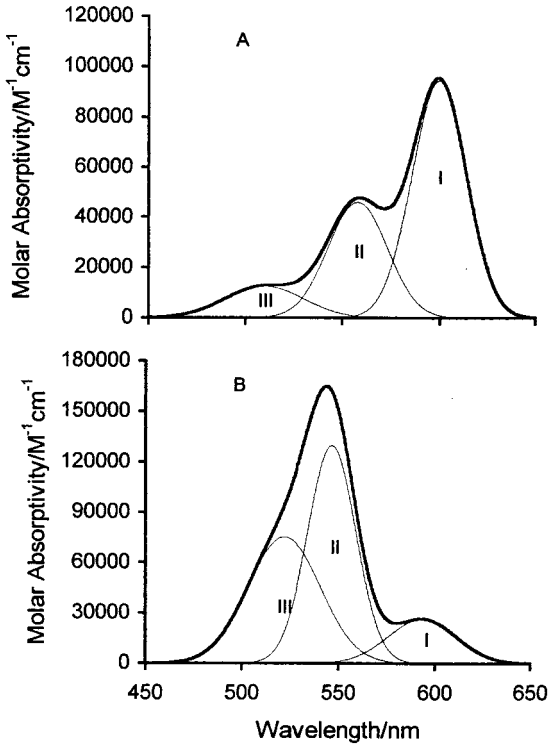

Figure 2. Reconstructed monomer (A) and dimer (B) absorption spectra of pinacyanol in water obtained from the theoretical values of molar absorptivities at any wavelength. Deconvolution was performed assuming a logarithmic-normal profile of the spectra.

nm with $\epsilon = (42.50 \pm 2.95)\cdot10^3$ M^{-1}·cm^{-1} indicate equilibrium between the two types of PIN molecules. Since the concentrations used were low, we assume that one of them is the monomer, and the other is the dimer. In such conditions, eq 4 holds.

The procedure used to obtain the molar absorptivities of dimer and monomer species leads to the determination of K_D, for which the deviation between the theoretical and the experimental spectrum attains its minimum. Accordingly, a K_D of $(350 \pm 5)\cdot10^2$ M^{-1} was obtained. The values of ϵ at any wavelength allowed us to obtain the individual absorption spectra of monomer and dimer species. Figure 2 shows the reconstructed absorption spectra of monomeric and dimeric PIN deconvoluted into a sum of Gaussian peaks. Figure 2A shows the deconvolution of the PIN monomer absorption spectrum into bands I, II, and III. Figure 2B shows the separation of the dimer spectrum, which also gave three Gaussian bands. These functions

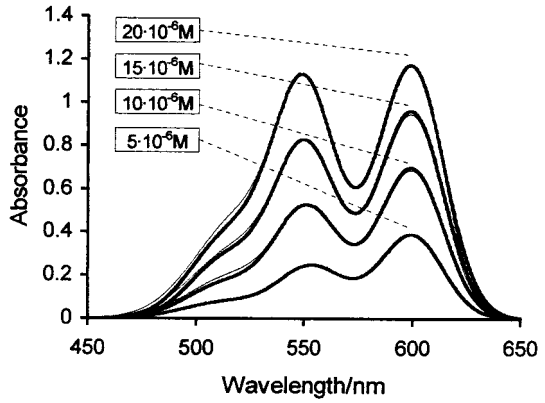

Figure 3. Comparison between the experimental spectra (thin line) and the theoretical spectra (thick line) for PIN at concentrations of $2.0\cdot10^{-5}$, $1.5\cdot10^{-5}$, $1.0\cdot10^{-5}$ and $0.5\cdot10^{-5}$ M.

Table 1. Amplitude (Molar Absorptivity), Area of the Curve, Center (λ_{max}), and Half-Bandwidth of the Bands Obtained from Gaussian Curve Fitting Analysis of the Absorption Spectra of the Monomer and Dimer of PIN

monomer	ϵ/M^{-1}·cm^{-1}	area/10^5	λ_{max}/nm	width/nm
band I	94,400	33.2	599.8	14.0
band II	46,000	17.5	557.9	15.2
band III	12,500	6.3	509.9	20.0
dimer				
band I	26,400	11.6	593.4	17.5
band II	129,700	40.6	546.6	12.5
band III	75,400	35.5	522.0	18.7

fit the spectral data (Figure 3). Table 1 shows the data obtained for each Gaussian band resolved. From Figure 2 and Table 1, we infer that band I prevails and that band III contributes to only about 10% of the area of the overall spectrum when PIN is present as a monomer. In contrast, bands II and III are higher when PIN is mainly in dimeric form. For instance, the ratio between the areas of the Gaussian band I and the sum of bands II and III is 1.39 when PIN is a monomer, and 0.15 when it is a dimer. Thus, the decrease in the intensity of band I at increasing dye concentrations, which was paralleled by a rise in the intensity of band II and the appearance of a more evident absorption band at 522 nm on the vibronic shoulder of the monomer, is a signature of dimer formation.

Interaction of PIN with DTAB. The interaction of micellar DTAB with PIN was studied at several surfactant concentrations ranging from 0.02 to 0.16 M and at three aqueous dye concentrations: $9.36\cdot10^{-6}$, $1.25\cdot10^{-5}$, and $1.73\cdot10^{-5}$ M. Figure 4 presents the absorption spectra of $1.73\cdot10^{-5}$ M PIN for a DTAB concentration from 0 to 0.16 M. Deconvolution of these spectra also afforded three Gaussian bands (graphs not shown). A sharp isosbestic point at 559.2 ± 0.2 nm with $\epsilon = (40.30 \pm 0.70)\cdot10^3$ M^{-1}·cm^{-1} points to an equilibrium between two spectrophotometrically distinguishable states of PIN, that is, free PIN and bound PIN in the micelles.[29] Such isosbestic point may indicate a 1:1 complex formation between the dye and the micelles.[4] When the surfactant concentration increased from 0.02 to 0.0.4 M, the absorption of band I augmented. The increase in absorbance values at increasing surfactant concentrations indicates that a larger number of the dye molecules are taken up by DTAB micelles. This trend has also been observed elsewhere.[30]

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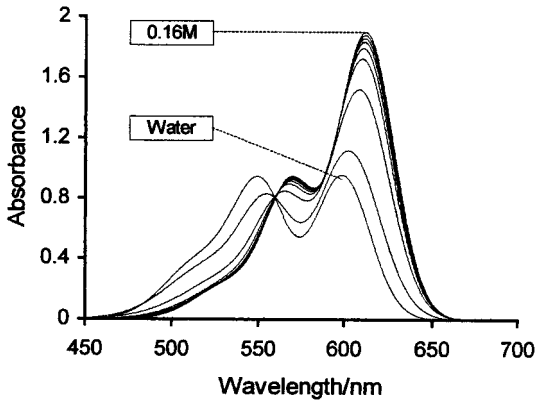

Figure 4. Visible absorption spectrum of $1.73 \cdot 10^{-5}$ M PIN in several concentrations of DTAB (from 0 to 0.16 M) at 25 °C.

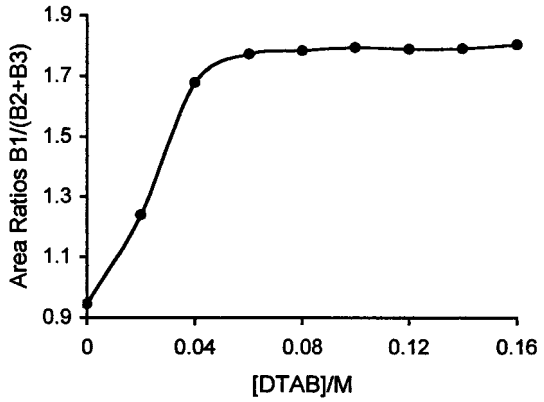

Figure 5. Ratio between the Gaussian band I and the sum of the Gaussian bands II and III of the deconvoluted spectrum as a function of DTAB concentration.

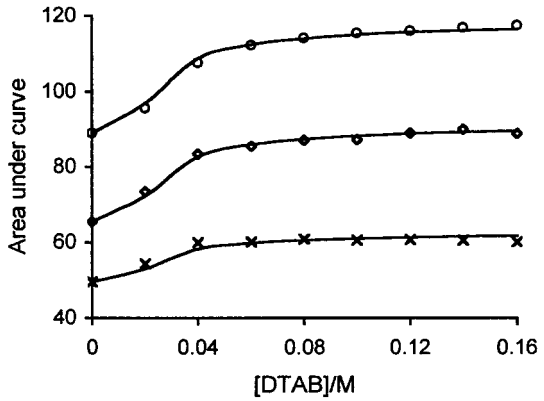

Figure 6. Plots of the area under the spectral curve (arbitrary units) as a function of the concentration of micelles at three PIN concentrations.

At 0.04 M, absorption seemed to reach a maximum. This hyperchromic effect was accompanied by a bathochromic shift due to the surfactant-dye interaction. The other bands also underwent a shift to the red, but at this stage, absorbance was attenuated. The shift to the red indicates that the PIN chromophore was then located in a less-polar (micellar) environment. The hydrocarbon core of any micelle has a dielectric constant of 2−5,[31] whereas a value of 69 was estimated for the interfacial region of a PIN-*n*-alkyl-trimethylammonium bromide surfactant system.[25] Both values differ from that of water (≈80). In the presence of micellar DTAB, PIN interacts with the micellar aggregate through the attraction of the noncharged aromatic quinocyanine ring present in the dye to the cationic headgroups of the surfactants on the micellar surface, that is, by means of a cation-π interaction.[25]

The similarity between the absorption spectrum of PIN in the presence of micellar concentrations of DTAB and the deconvulated spectrum of the monomer supports that PIN incorporates into the micelle as a monomer. We thus suggest a new equilibrium:

$$\text{dimer} \overset{K_D}{\longleftrightarrow} \text{monomer(bulk)} \overset{K_A}{\longleftrightarrow} \text{monomer(micellar)} \quad (7)$$

Therefore, the presence of micelles produces a shift to the right and, as in similar dyes,[5,8,23,32] the conversion of monomers into dimers at increasing DTAB concentrations was less extensive.

Figure 5 shows the variation in the area ratios between band I and bands II and III for a PIN concentration of $9.36 \cdot 10^{-6}$ M. This ratio is a sensitive parameter to evaluate the influence of an agent on the dimerization process. The ratio between areas increased from water to 0.06 M DTAB. From this surfactant concentration, well above the critical micellar concentration in water (0.015 M), the ratio was almost unchanged and the system dye surfactant seemed to have reached the equilibrium. The same pattern was found for the other two PIN concentrations.

Determination of the Micelle Association Constant. Since dye molecules are distributed between bulk solvent and micellar phases, the corresponding equilibrium is given by the micelle association constant, K_A, which is calculated from eq 5. In this eq., we used the area under the curve instead of the absorbance because the relation area-dye concentration is linear ($r > 0.997$), whereas absorbance (or molar absorptivity) does not obey the Lambert−Beer law. The experimental parameters are $Area_{obs}$, $Area_w$, surfactant concentration, and cmc. A nonlinear curve fitting of a plot of $Area_{obs}$ vs M (Figure 6) affords the values of the constant as well as the number of surfactant molecules associated with one micelle. With this approach, we obtained $K_A = 2160 \pm 100$ M^{-1} and $n \approx 1$. When data were fitted to equations that presuppose a 1:1 complex, like the Scott and Hildebrand equations,[33] an almost identical value of K_A was obtained. This corroborates that the maximum number of dye molecules that each micelle can accommodate is one. A free energy change of -19 kJ·mol^{-1} ($\Delta G = -RT\ln K_A$) corresponds to the association constant obtained. This supports our previous finding that the attractive effect, arising from the cation-π interaction, is sufficiently intense to overcome the Coulombic repulsion between positive species.

Once the K_A value is known, we can calculate the fraction of micellized (f_{mic}) and nonmicellized (f_w) PIN from

$$f_{mic} = \frac{K_A[M]}{1 + K_A[M]} \quad (8)$$

$$\text{and } f_w = \frac{1}{1 + K_A[M]} \quad (9)$$

respectively.

The values of micellar fraction of PIN are listed in Table 2. The nonmicellized PIN is the amount of dye molecules

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Table 2. Fraction of Micellized PIN at Various DTAB Concentrations

[DTAB]/M	fraction
0	0
0.02	0.223
0.04	0.557
0.06	0.657
0.08	0.705
0.10	0.733
0.12	0.751
0.14	0.764
0.16	0.774

present in the bulk solution, in a proportion between monomers and dimers that can be determined by the dimerization constant, K_D.

In the inner and interfacial regions of the micelle, the dielectric constant is different from that in the aqueous bulk. Thus, the molar absorption coefficients vary and the spectrum of the monomer into the micelle differs from that in solution. The experimental spectra of PIN in the presence of micellar DTAB can be considered the sum of the spectra of monomer and dimer into the bulk and the spectrum of PIN into the micelle. From eq 8 and 9 it is possible to determine the fraction of PIN in micellar form (f_{mic}) and in the bulk of solution (f_w). Using the dimerization constant and f_w allows us to know the amount of dye as monomer and as dimer in the bulk, as well as the corresponding theoretical spectra. In this way, the micellar spectrum of the dye can be obtained by subtracting from the experimental spectrum the spectra obtained for the dimer and monomer into the bulk. Figure 7 displays the spectrum of micellized PIN obtained by spectrum difference. This spectrum was also resolved in three Gaussian bands centered at 612.8 (ϵ = 127,800 $M^{-1}\cdot cm^{-1}$), 568.3 (ϵ = 56,600 $M^{-1}\cdot cm^{-1}$), and 533.7 nm (11,300 $M^{-1}\cdot cm^{-1}$), and its shape corresponds to that of the monomer in solution (Figure 2A), with a bathochromic shift of bands because the dielectric constant in the micelle is lower than in water. This further supports that the dye is incorporated into the micelle as a monomer.

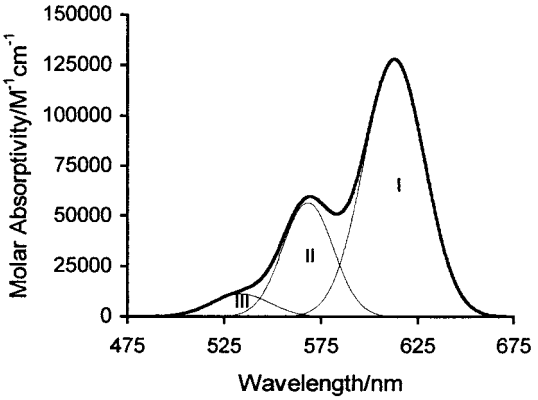

Figure 7. Calculated visible absorption spectrum of micellized PIN obtained by difference of spectra with the three deconvoluted bands.

Acknowledgment. R.S. is the recipient of a grant from the Generalitat de Catalunya (FI 00337 UB).

LA010463Y