SOLVENT EFFECTS ON THE ELECTRONIC ABSORPTION AND FLUORESCENCE EMISSION SPECTRA OF MEROCYANINE 540 - A BIOLOGICAL PROBE[1]

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Abstract: The electronic absorption and fluorescence spectra of merocyanine 540 (MC-540) were determined at 298 K in several solvents of different polarity (dioxane, toluene, triethylamine, diethyl ether, chloroform, methyl acetate, pyridine, acetone, ethanol, methanol, nitromethane, acetonitrile, and dimethyl sulfoxide). For the first time, the ground-state dipole moment of MC-540 was measured in dioxane-dimethylformamide (15 : 1, v/v) at 298 K. Solvent effects were evaluated using the solvatochromic shift method (Bakhshiev and Kawski-Chamma-Viallet correlations). The spectral blue shifts observed with increasing solvent polarity indicate a slightly lower value of the dipole moment in the first excited singlet state as compared with the ground-state dipole moment. The application of the Kamlet-Abboud-Taft polarity parameters to the electronic spectral data of MC-540 is discussed.

Introduction

Merocyanine 540 (MC-540, 62796-23-0) is an anionic, lipophilic fluorescent dye which has been predominantly used as a photosensitizer in photographic emulsions (1) and as an external biological probe in studies of a number of artificial and cellular membranes (2-8). Also, MC-540 has been utilized in biomedicine in the photodestruction of various human cancer cells (9). Recently, it has found clinical application in the photo-chemotherapeutic treatment of patients with metastatic neuroblastoma (10,11). One of the interesting physical features of MC-540 is that it exhibits electrochromism (i.e., change in electronic absorption and/or emission spectra due to an external electric field) (12). Because of its structure, the various physical and chemical properties of MC-540 depend on the polarity of the solvent. Several solvent polarity-related spectral quantitative studies (including a plot of the absorption maximum wavenumber against the macroscopic dielectric

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constant of the medium) have been reported (3,13). The general pattern of such correlations is a partially linear hypsochromic shift of the spectral data with increasing polarity of the solvent. However, it is important to point out that these correlations include only a limited number of pure solvents or various alcoholic mixtures. Recently, the ground-state and excited singlet-state dipole moments of MC-540 have been estimated from solvent-induced spectral-shift correlations, based on only one type of a solvent function, and obtained in a series of aliphatic nitrile solvents (13). Finally, only one experimental ground-state dipole moment value has been reported for MC-540 (2). The above-mentioned studies of MC-540 have prompted us to publish our results. In the present contribution, we wish to present our data on the effect of polar and nonpolar solvents (dioxane, toluene, triethylamine, diethyl ether, chloroform, methyl acetate, pyridine, acetone, ethanol, methanol, nitromethane, acetonitrile, and dimethyl sulfoxide) on the electronic absorption and fluorescence spectra of the fluorescent probe MC-540. Furthermore, the usefulness of the solvatochromic method for the evaluation of the lowest excited singlet-state dipole moment of MC-540 will be demonstrated. The experimental ground-state and the first excited singlet-state dipole moments of MC-540 have been determined and compared with a theoretical value calculated as a vector sum of the π-component (PPP method) and the σ-component (σ-bond contributions). In order to evaluate the different types of solvent effects, the Kamlet-Abboud-Taft equation has been applied to our spectral data. More detailed results will be presented in a follow-up full-size paper.
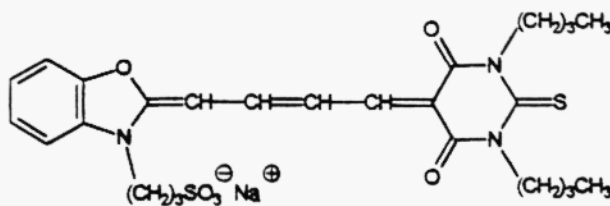
MC-540

Experimental

Merocyanine 540 (MC-540) was purchased from Sigma Chemical Company, St. Louis, MO, and recrystallized from ethanol before use. Spectroscopic grade solvents were used for the measurements of the spectra and the determination of the dipole moments. The ground-state dipole moment of MC-540 was obtained in dioxane-dimethylformamide (15 : 1, v/v) as previously described (14). The experimental solid-state density of MC-540 (δ_4^{23} = 1.316) was determined pycnometrically at 296 K in the form of a suspension of MC-540 (300 to 900 mg) in kerosene. The value of the Onsager cavity radius of MC-540 (a_0 = 5.56 x 10^{-8} cm) was calculated

from its molecular volume using our experimental value of δ. Electronic absorption spectra were recorded at 298 K in different solvents on a Cary 118 spectrophotometer equipped with a thermostatted cell holder. Fluororometric experiments (fluorescence emission and excitation spectra) were carried out at the same temperature on a Perkin-Elmer LS-50 spectrophotofluorometer. The wavelengths of all absorption and fluorescence maxima were determined with an accuracy of ± 1 nm. Because MC-540 forms aggregates in water and in organic solvents (3,15), all absorption and fluorescence spectra were obtained at low concentrations (5.44 x 10^{-7} mol. l^{-1}) using 10-mm quartz cells. The above concentration was used because a concentration study had indicated the absence of any significant changes of the homothetic shape of the MC-540 absorption and excitation spectra as well as of its molar absorption coefficient and the position of the emission maximum within the 10^{-6}-10^{-7} mol.l^{-1} concentration range. The excited singlet-state dipole moments were evaluated from the solvatochromic shifts using the Bakhshiev and Kawski-Chamma-Viallet formulas (14). The solvent functions F_1 and F_2 were calculated using the dielectric constant and index of refraction values of solvents as previously described (14). In order to determine the respective contributions of the polarity of the solvents and of their hydrogen-bond donor (HBD) and acceptor (HBA) abilities on the photophysical properties of MC-540, the simplified form of the Kamlet-Abboud-Taft equation (16) has been applied to our electronic absorption and fluorescence spectral data.

Results and Discussion

In all organic solvents under study, MC-540 absorption spectra exhibit a band of maximum absorbance with additional maxima at shorter wavelengths. The typical shape of the absorption curves can be attributed to the visible $\pi \rightarrow \pi^*$ electronic transition of MC-540 as commonly observed with many types of planar polyheteroaromatic dyes. In all solvents used, the MC-540 fluorescence excitation spectrum is homothetic to its absorption spectrum and an identical emission spectrum is obtained when exciting over any band of the absorption spectrum of MC-540 (Fig. 1). These observations allow us to assign the longest-wavelength absorption band to the 0-0 $\pi \rightarrow \pi^*$ electronic transition, and the shorter-wavelength shoulders to vibrational components arising from simultaneous excitation of atoms in the conjugated tetramethine chain. These results confirm that only one major emitting species is present in the solutions under study, i.e., the monomeric form of MC-540. The spectral characteristics of MC-540 are summarized in Table 1. In agreement with the above assignment, the molar absorption coefficient of the band of maximum intensity is greater than 1.0 x 10^5 l.mol^{-1}. cm^{-1} as normally found for analogous cyanine dyes (17). When going from chloroform to methanol, weak blue shifts of the absorption (521 cm^{-1}) and fluorescence emission (360 cm^{-1}) maxima are observed. Such blue shifts with increasing solvent polarity, usually called negative solvatochromism, occur when the ground state is more polar than the first excited singlet state (i.e., when the ground-state dipole moment is higher than the first excited singlet-state dipole moment). To determine the first excited singlet-state dipole moment of MC-540, the Stokes shift values, $(\tilde{\nu}_A - \tilde{\nu}_F)$ and $(\tilde{\nu}_A + \tilde{\nu}_F)/2$, were plotted against the solvent functions, F_1 and F_2, accord-
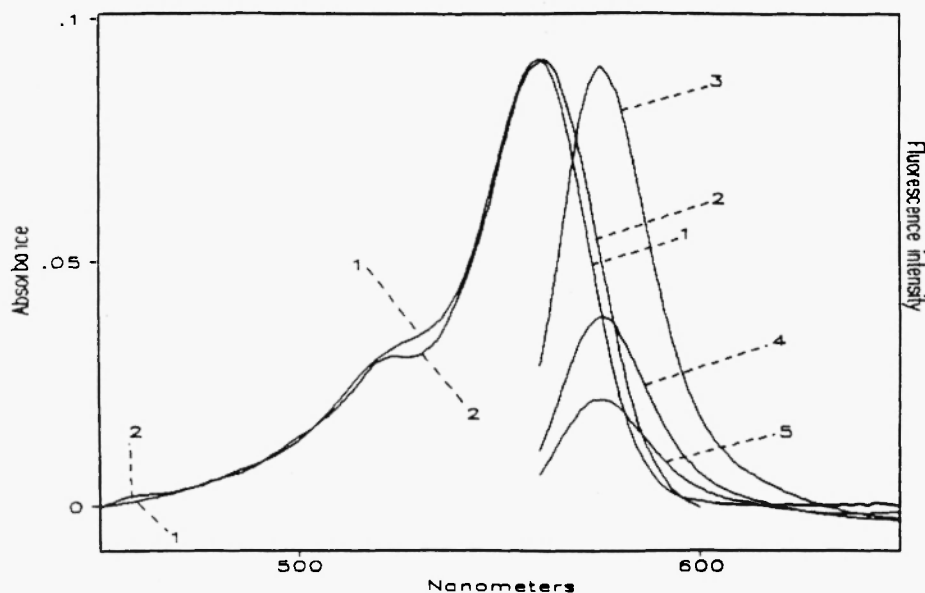
Fig. 1. Comparison of the absorption (curve 1), excitation (curve 2, λ_{em} 620 nm), and emission (curves **3**, **4**, and **5** at λ_{ex} 550, 525, and 480 nm, respectively) spectra of merocyanine 540 (5.44 x 10^{-7} mol.l^{-1}) at 298 K in ethanol.

ing to the Bakhshiev and Kawski-Chamma-Viallet equations (14). The correlation coefficients, *r*, are higher than 0.93, indicating a good linearity for most solvents. Because of the specific MC-540-solvent interactions and/or the approximations used in the solvatochromic method, some solvents show excessive deviations and have not been included in the correlations. To the best of our knowledge, our experimental ground-state dipole moment, μ_g = 5.25 ($\pm$0.43) D, is the first one reported for MC-540. It compares relatively well with the values obtained indirectly in water-membrane interface (8.6 $\pm$ 2.3 D) (2) or roughly estimated in aliphatic nitriles and alcohols (9.6 D) (13), taking into account the large differences in the individual experimental procedures. Furthermore, our experimental value is closer to our theoretical value of 4.70 D (18). A good agreement is also obtained between the first excited singlet-state dipole moment values (μ_e) values determined from the Bakhshiev (4.23 D) and Kawski-Chamma-Viallet (4.47) equations. Again, these values compare favorably with the previously estimated value of the first excited singlet-state dipole moment, μ_e = 5.2 D (13). Our results indicate that the dipole moment of MC-540 is slightly lower in the first excited singlet state than in the ground state, thus supporting the weakly negative solvatochromic behavior observed for this compound. We have found that the decrease of the dipole moment upon excitation is somewhere between 0.8 and 1.1 D. This difference can be favorably compared with the $|\Delta\mu|$ = 0.46 ($\pm$0.05) D value estimated from electrochromic measurements for MC-540 incorporated into phospholipid layers (12). This decrease of the polarity of the first excited singlet state as compared to the ground state may be attributed to the slightly more stabilized weakly polar resonance structure of MC-540 in the ground state and/or to contraction of the dipole

Table 1. Wavenumbers of the absorption ($\tilde{\nu}_A$) and fluorescence ($\tilde{\nu}_F$) maxima of merocyanine 540 (5.44×10^{-7} mol.l^{-1}) in various solvents (in cm^{-1})

Solvent	$\tilde{\nu}_A$	$\tilde{\nu}_F$	$\epsilon \times 10^{-5}$ *
Dimethyl sulfoxide	17715	17241	1.42
Acetonitrile	17905	17422	1.82
Nitromethane	17825	17361	1.64
Methanol	18034	17483	1.51
Ethanol	17857	17361	1.66
Acetone	17857	17391	1.82
Chloroform	17513	17123	1.74
Toluene	17544	17123	1.83
Dioxane	17668	17241	2.00
Methyl acetate	17815	17271	1.92
Diethyl ether	17699	17391	1.00
Triethylamine	17699	17271	1.61
Pyridine	17544	16892	1.44

*In l.mol^{-1}.cm^{-1}.

in the excited state. In the latter hypothesis, a change from the all-*trans* configuration to a *cis*-configuration of the tetramethine chain would occur upon excitation of MC-540. Also, it has to be pointed out that our values of the experimental dipole moments in both electronic states are much lower than the dipole moment of about 35 D estimated for the zwitterionic forms of a similar merocyanine dye using MM2 molecular mechanics calculations (19). These results suggest that, while the zwitterionic resonance structures do not play an important role in the ground and excited singlet states of MC-540, the weakly resonance-stabilized form contributes significantly to the electronic structure of both states (19). In order to elucidate the nature of the specific solute-solvent interactions, the electronic absorption ($\tilde{\nu}_A$) and fluorescence ($\tilde{\nu}_F$) maxima wavenumbers were plotted against the following parameters using the simplified form of the Kamlet-Abboud-Taft equation (16): solvent polarity/polarizability (π^*), solvent HBD ability (α), and solvent HBA ability (β). The following linear correlations were obtained:

$$\tilde{\nu}_A = 17490 + 361 (\pm 25) \, \pi^* + 180 (\pm 17) \, \alpha + 221 (\pm 9) \, \beta \quad (r = 0.998; n = 7) \tag{1}$$

$$\tilde{\nu}_F = 17123 + 219 (\pm 2) \, \pi^* + 135 (\pm 3) \, \alpha + 122 (\pm 1) \, \beta \quad (r = 0.919; n = 6) \tag{2}$$

The majority of the solvents obeyed these multiparameter correlations, with the exception of dimethyl sulfoxide, pyridine, toluene, and chloroform. The relationships between the $\tilde{\nu}_A$ and $\tilde{\nu}_F$ experimental and

calculated values (cf. Eqs. 1 and 2) were linear. The positive sign of the polarity/polarizability coefficients (s) implies that increasing solvent polarity/polarizability parameter (π^*) leads to a hypsochromic shift of $\bar{\nu}_A$ and $\bar{\nu}_F$. This suggests that the excited singlet state of MC-540 is less stabilized with increasing solvent polarity. The above conclusion is in agreement with the slightly lower dipole moment of MC-540 observed in the excited singlet state when compared with the ground-state value. The solvent HBD and HBA ability coefficients (a and b, respectively) also present positive but smaller values thus indicating that solvents with increasing HBD and HBA abilities shift $\bar{\nu}_A$ and $\bar{\nu}_F$ hypsochromically. This result demonstrates the existence of multiple hydrogen-bonding, solute-solvent interactions. It is also worth noting that the values of the a and b coefficients are significantly smaller than the values of the s coefficients (cf. Eqs. 1 and 2). This means that in the excited singlet state of MC-540, solute-solvent interactions are dominated by nonspecific dipole-dipole forces rather than by hydrogen-bonding effects.

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