

DIMERS OF MEROCYANINE 540 I. IN AQUEOUS SOLUTIONS

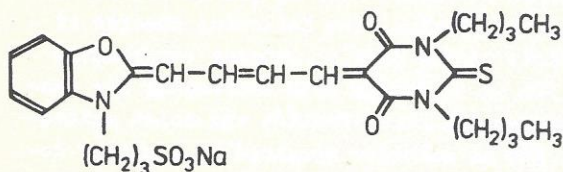
L. ŠIKUROVÁ, I. HABÁN, D. CHORVÁT

Comenius University, Department of Biophysics MFF, Bratislava

A formation of dimers of the dye merocyanine 540 in aqueous solutions in dependence on the dye concentration was investigated. The absorption peak of dimers is at wavelengths 500 nm and monomers at 535.5 nm, respectively. The M-540 absorption spectra show two isosbestic points (523.5 nm, 560.5 nm), which confirms the existence of two coloured species (monomers and dimers). The excitation fluorescence spectrum of M-540 in water shows only one peak originating in fluorescent monomers. Aqueous solutions of M-540 dye are not stable, their spectra show changes with time: With increasing time after preparation of aqueous solutions, intensity of both peaks is reduced and new band at 386 nm appeared.

Introduction

Merocyanine 540 (abbreviated M-540) is a fluorescent dye widely used since the year 1973 /1/ in the biophysical and medical research /4,6,7,9/. Spectral properties of M-540 dye (see structure below)



are distinctively sensitive to polarity of the environment showing hypsochromic solvatochromic behaviour /3,5,8/. Besides, M-540 spectra are dye concentration dependent in water solution and in biological or model membranes /2,3,11/. These spectral changes have been attributed to aggregation of the dye molecules in water to form dimers or higher polymers under the influence of the strong dispersion forces associated with the high polarizability of the chromophoric chain /13/.

The aqueous M-540 spectra are reported in papers /4,5,8,10/ but

only for one dye concentration. LELKES and MILLER /3/ presented concentration dependence of the relative increase in the dimer absorption band of M-540 in aqueous solutions. The total absorption spectra of M-540 in water at varying dye concentration and also the fluorescence excitation spectra have not yet been published. Our work presents concentration changes of aqueous M-540 absorption spectra and fluorescence excitation spectra with the aim of identifying the coloured species - monomers, dimers or higher aggregates.

Materials and methods

Merocyanine 540, molecular weight 569.68, was obtained from Eastman Kodak, Co., and was used without further purification. Bidistilled water ($\text{pH} = 6.2 \pm 0.2$) was used for solution preparation. M-540 stock solution of 2×10^{-4} mol/l in water was freshly prepared daily. Aliquots of the stock solution were added to each aqueous solution to yield the desired concentration. M-540 concentrations varied in the range of (2×10^{-8} - 1.75×10^{-4}) mol/l. The visible absorption spectra were recorded by SPECORD M 40 spectrophotometer. The corrected fluorescence excitation spectra were recorded by PERKIN-ELMER LS5 spectrofluorimeter. All measurements were carried out at temperatures of $22 \pm 2^\circ\text{C}$. The samples were protected from light.

Results and discussion

FIG. 1 shows visible electronic absorption spectra of aqueous solutions of M-540 dye at varying dye concentration. The spectra show two absorption peaks at 535.5 ± 1.0 nm and at 500-505 nm. With increasing M-540 concentration, the following changes of the spectra were detected: i/ The peak at shorter wavelengths was blue-shifted from 505 nm to 500 nm with increasing dye concentration. ii/ With increasing concentration the molar extinction coefficient ϵ at 500 nm enhanced compared to a slight decrease at 535.5 nm. These concentration dependent changes characterize the M-540 aggregation process. The presence of two isosbestic points (523.5 nm; 560.5 nm) in absorption spectra (FIG. 1) reflects the existence of two various dye species (monomers and dimers). Higher aggregates were not observed in the concentration range used. The longer wavelength band can be attributed to dye monomers and the shorter wavelength band to dimers. Our data for peak wavelengths are in agreement with the analogous data in /3,4,5,8,10/. A decrease of the dye concentra-

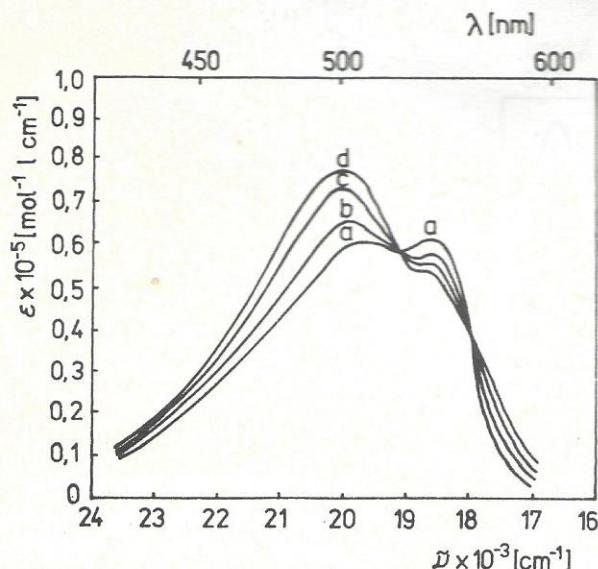


FIG. 1 Visible absorption spectra of M-540 in water. Concentrations a/ (2×10^{-8} - 1×10^{-6}) mol/l, b/ 3.5×10^{-5} mol/l, c/ 9×10^{-5} mol/l, d/ 1.75×10^{-4} mol/l

tion below 1×10^{-6} mol/l does not alter the M-540 spectrum, in the case of freshly prepared samples. So the absorption spectrum of freshly prepared M-540 solutions at infinite dilution also exhibits two absorption peaks at 500 nm and 535.5 nm with approximately the same value of ϵ equal to 6×10^4 mol $^{-1}$ cm $^{-1}$. Even at infinite dilution, this spectrum cannot be considered to be a monomeric spectrum because the excitation fluorescence spectrum of M-540 in water (FIG. 2) shows only one peak originating in fluorescent monomers whereas the dimer fluorescence is negligible.

The absorption spectra changed with time (FIG. 3): With increasing time after preparation of aqueous solutions the intensity of both peaks is reduced. After two months the bands at 500 nm and 535 nm disappeared in favour of a new band at 386 nm. This fact shows that the aqueous solutions of M-540 dye at concentrations of 10^{-6} mol/l and less are not stable and therefore are not suitable to research applications because it is difficult to make accurate measurements of dilute aqueous dye solutions

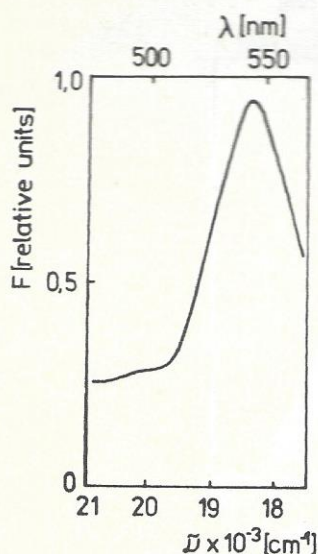


FIG. 2 Corrected excitation fluorescence spectrum of M-540 in water ($c = 3 \times 10^{-4}$ mol/l)

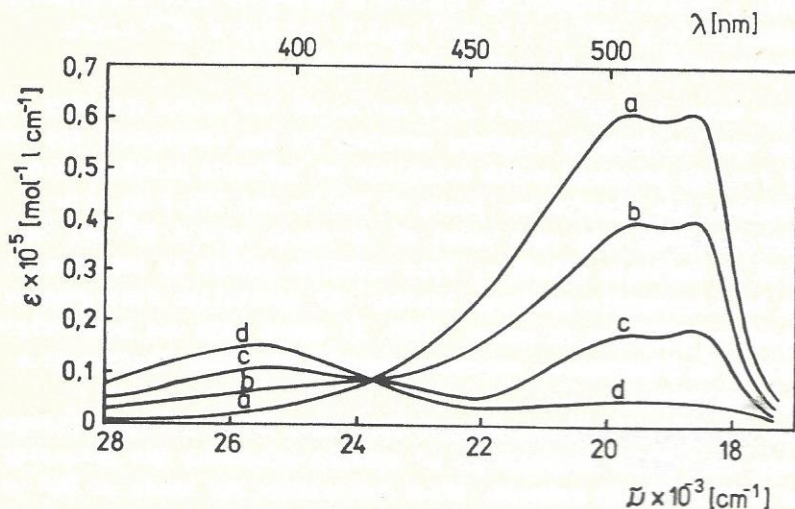


FIG. 3 Changes in absorption spectra of M-540 in aqueous solution ($c = 2 \times 10^{-7}$ mol/l) as a function of time. Curves a/ b/ c/ d/ represent spectra for 0, 5, 18, 60 days, respectively, after solution preparation

even when precautions are taken to exclude acting light during preparation of the solutions. Furthermore freshly prepared solutions are required to obtain reproducible results of M-540 dye in water.

References

- /1/ DAVILA, H. V., SALZBERG, B. M., COHEN, L. B., Nature New Biology 241 (1973) 159
- /2/ DRAGSTEN, P. R., WEBB, W. W., Biochemistry 17 (1978) 5228
- /3/ LELKES, P. I., MILLER, I. R., J. Membr. Biol. 52 (1980) 1
- /4/ MONTICH, G. G., BUSTOS, M. M., CUMAR, F. A., Chem. Phys. Lipids 38 (1985) 319
- /5/ POHL, G. W., Z. Naturforsch. 31c (1976) 575
- /6/ ROSS W. V., SALZBERG, B. M., COHEN, L. B., DAVILA, H. V., Biophys. J. 14 (1974) 983
- /7/ SIEBER, F., SPIVAK, J. L., SUTCLIFFE, A. M., Proc. Natl. Acad. Sci. USA 81 (1984) 7584
- /8/ ŠIKUROVÁ, L., JANÍKOVÁ, T., Studia biophysica 118 (1987) 189
- /9/ SMITH, J. C., GRAVES, J. M., WILLIAMSON, M., Arch. Biochem. Biophys. 231 (1984) 430
- /10/ TASAKI, I., WARASHINA, A., Photochem. Photobiol. 24 (1976) 191
- /11/ WAGGONER, A. S., GRINVALD, A., Ann. N. Y. Acad. Sci. 303 (1977) 217
- /12/ WARASHINA, A., TASAKI, I., Proc. Japan Acad. 51 (1975) 610
- /13/ WEST, W., PEARCE, S., J. Phys. Chem. 69 (1965) 1894

Было исследовано возникновение димеров красителя М-540 в водных растворах в зависимости от концентрации красителя. Полоса поглощения димеров М-540 - 500 нм и мономеров - 535.5 нм. На спектрах поглощения М-540 находятся две изобестические точки 523.5 нм; 560.5 нм, что подтверждает существование двух сортов красителя М-540 /мономеров и димеров/. С возрастанием времени после приготовления водных растворов интенсивность обоих полос уменьшалась и появилась новая полоса при 386 нм. Водные раствора красителя М-540 не являются стабильными, их спектры во времени меняются. Спектр возбуждения М-540 в воде имеет только одну полосу, которая отвечает флуоресцирующему мономеру.

Received on 18. 11. 1987, in final version on 21. 6. 1988

Dr. L. ŠIKUROVÁ, Department of Biophysics MFF, Comenius University, Mlynska dolina F 1, CS-842 15 Bratislava, Czechoslovakia