

EFFECT OF SOLVENTS ON THE ABSORPTION SPECTRA OF MEROCYANINE 540

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Electronic absorption spectra of merocyanine 540 molecules were measured in the visible region for twenty one solvents or solvent mixtures of differing polarity. A blue solvatochromic shift of the measured spectra was observed with the increasing polarity of the solvent and the quantitative dependence between the position of the absorption peak and dielectric constant of the solvent was established. This calibration of solvatochromic behaviour of merocyanine 540 was used for the probing of the local polarity of phospholipid liposomes.

Introduction

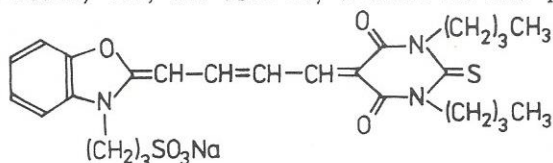
Various fluorescent dyes have been largely used as a tool of biophysical and biomedical research. Among them, a frequently employed probe is merocyanine 540 (abbreviated as M-540). This dye was previously applied widely for monitoring of action potentials on some biological preparations /2,3,4,12,14/. Because of photodynamic damage this application has recently been dropped. Later on M-540 was applied for additional studies which have shown that this dye is a sensitive probe of structural modifications in lipid membranes /5/. POHL /10/ and LELKES and MILLER /5/ discussed the use of M-540 as an indicator of phase transition in lecithin membranes. Studies published in /8/ have shown that M-540 distinguishes discrete domains in the plasma membranes of various cells and biological samples. WILLIAMSON et al. /15/ have proved that M-540 is able to sense the degree of lipid packing of bilayers. In /11/ the authors assessed the ability of M-540 to monitor the charge separation in submitochondrial particles. MONTICH et al. /7/ used M-540 to investigate the particular lipid composition (the portion of glycosphingolipids) in liposomes and to study a penetration of M-540 in monolayers of various lipids.

The above-mentioned measurements showed that the probe M-540 appears particularly sensitive in one membrane system, but it is not necessarily sensitive in another membrane /6/. The reason of

this behaviour is not yet explained. Probably, it is the reflection of the dye ability to interact with distinct membrane systems. This assumption is supported also by the fact that M-540 ranks among a large family of chain dyes - benzoxazol merocyanines - with strongly solvent dependent optical characteristics /1,9/. With the aim to calibrate the solvatochromic behaviour of M-540 dye, we present here the systematic studies of the solvent effects on M-540 electronic absorption spectra. We hope that our results will help to improve the physical-chemical interpretation of M-540 spectra in various membranes.

Materials and methods

In our experiments merocyanine 540 (M-540) was used as a fluorescent probe (see structure below). This dye we obtained from Eastman Kodak, Co., and used it, without further purification.



For solvatochromic studies the following solvents were used: bi-distilled water ($\text{pH} = 6.2 \pm 0.2$), ethylene glycol, methanol, ethanol, n-butanol, isobutanol, acetone, chloroform, benzene, p-xylene, carbon tetrachloride, n-hexane, n-decane. Solvents of spectroscopic quality were employed and were obtained from Lachema, Brno, except for isobutanol (Reachim, USSR), n-hexane, n-decane (Merk - Darmstadt, West Germany) and ethylene glycol (Fluka, Buchs, Switzerland). In order to cover the whole range of polarity, homogenous solvent mixtures of methanol - water, benzene - methanol, chloroform - methanol and carbon tetrachloride - methanol were used. Fresh M-540 solutions were prepared daily.

Phospholipids employed in our experiments were synthetic lecithins obtained from Fluka AG: L- β , γ -dimyristoyl- α -lecithin (DML), L- β , γ -dipalmitoyl- α -lecithin (DPL), L- β oleoyl- γ -palmitoyl- α -lecithin (OPL). We also used egg lecithin (EL) prepared at the Charkow State University. All these lecithins were employed in the preparation of the "alcohol" liposomes according to the standard method of VLADIMIROV and DOBRETISOV /13/. The M-540 concentrations in all solutions were in the range of $(5 \times 10^{-7} - 3 \times 10^{-5})$ mol/l. The spectra were recorded by spectro-

photometer SPECORD UV VIS (Zeiss, Jena) within the $(14 - 30) \times 1000 \text{ cm}^{-1}$ region.

Quartz cuvettes with 0.5 cm optical pathway were used for measurements of M-540 absorption spectra in lecithin liposomes and another pair of cuvettes 1 cm thick for spectral measurements of M-540 in pure solvents or solvent mixtures.

All sample preparations and measurements were carried out at the temperature of $(19 \pm 2)^{\circ}\text{C}$.

Results and discussion

The systematic investigation of electronic absorption spectra of merocyanine 540 was made in various pure solvents of different polarity, in solvent mixtures and also in model phospholipid membranes. It was found that M-540 is soluble in solvents with sufficient polarity, such as water, ethylene glycol, methanol, ethanol, isobutanol, n-butanol, acetone and chloroform. In organic solvents with very low polarity, such as benzene, n-hexane, n-decane, p-xylene and carbon tetrachloride, it is practically insoluble. If a small volume of the polar solvent such as methanol (TAB.) is added to benzene, carbon tetrachloride or p-xylene facilitates the solubilization of the dye.

The visible electronic absorption spectra of the dye M-540 obtained in some pure solvents are shown in FIG. 1. In all visible absorption spectra of M-540 in solvents differing in their polarity, two peaks were observed. The peak at longer wavelengths was marked as A peak (monomer peak /5,12/) and the peak at shorter wavelengths as B peak (dimer peak /12/). In the range of the concentrations used ($5 \times 10^{-7} - 3 \times 10^{-5}$) mol/l no shift of A peak due to concentration changes was observed. But the B peak was shifted by concentration changes¹. From FIG. 1 it is evident that the shape and the position of the absorption peaks in M-540 visible spectra are strongly dependent on the polarity of the solvent, this means that M-540 exhibits solvatochromic behaviour. The absorption spectrum of the examined probe is blue shifted as the solvent polarity is increased - hypsochromic solvatochromism. M-540 molecules show an overall shift of 36.4 nm while passing from benzene - methanol (100 : 1) mixture to water. This corres-

¹detailed analysis in: ŠIKUROVÁ, L., HABAN, I.: Dimers of Merocyanine 540 (in preparation)

ponds to the difference in transition energy of 0.14 eV. The observed blue absorption shift corresponds to higher absorption transition energies with increasing solvent polarity. This spectral shift can be attributed to the changes in electronic structure of the dye /6/. The observed blue shift in M-540 spectra confirms, according to Brooker's ideas /1/, that M-540 is a dye of intrinsically high polarity. The values of λ_A are summarized in TAB., together with the solvent polarity parameter - dielectric constant ϵ_r (the dielectric constant has been proved as the sufficient solvent polarity parameter in these measurements). Our data for some of the pure solvents (ethylene glycol, methanol, ethanol, n-butanol, chloroform) are in

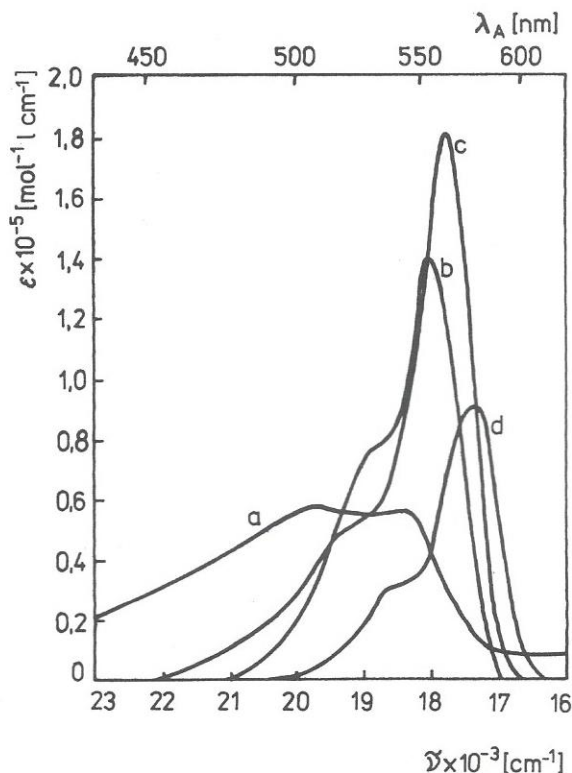


FIG. 1 ~~Chemical structure and~~ absorption spectra of M-540 in some solvents a) water (bidestilled pH = 6.2 $\pm$ 0.2), b) methanol, c) n-butanol, d) chloroform (concentration 1×10^{-5} mol/l).

TAB. 1 Absorption maxima of M-540 in solvents and lecithin liposomes

N°	Solvent	Dielectric constant at 25°C ϵ_r	Absorption maximum of A peak (nm) λ_A	Absorption energy of A peak (eV) E_A
1	water (pH = 6.2)	80.3	537.6	2.318
2	ethylene glycol	37.2	557.0	2.227
3	methanol	33.6	556.7	2.228
4	ethanol	25.5	560.2	2.214
5	acetone	20.7	562.4	2.206
6	isobutanol	17.9	564.3	2.199
7	n-butanol	17.1	565.0	2.196
8	chloroform	4.8	571.1	2.172
9	benzene - 100 : 1	2.6	574.0	2.161
10	methanol 20 : 1	3.6	572.8	2.166
11	10 : 1	5.1	569.5	2.179
12	2 : 1	12.7	566.5	2.190
13	carbon tetrachloride - methanol 1 : 4	27.3	559.9	2.210
14	chloroform - 1 : 1	19.2	563.1	2.203
15	methanol 5 : 1	9.6	567.8	2.185
16	10 : 1	7.4	568.2	2.184
17	water - 5 : 1	72.5	540.5	2.295
18	methanol 3 : 1	68.6	542.3	2.288
19	2 : 1	64.7	544.7	2.278
20	1 : 1	56.9	548.2	2.263
21	1 : 2	49.1	551.8	2.248
22	1 : 3	45.3	553.1	2.243
23	1 : 5	41.5	555.0	2.235
	Liposomes			
24	OPL		569.5	2.178
25	EL		570.8	2.174
26	DML		571.0	2.173
27	DPL		572.1	2.169

good agreement with those published in /5,7,10/. But the value of λ_A for M-540 in water, published in different literature, exhibits slight differences: 532 ± 2 nm /6,10/, 535 nm /7/, 537.6 nm /our result/. These deviations can be attributed to i) the different value of the temperature ii) the different value of pH of water used. Experimental values of the M-540 wavelength λ_A (for A peak) are plotted as a function of dielectric constant ϵ_r of the solvents used (FIG. 2). This plot exhibits linearity with a negative slope above the range of ϵ_r values

4.8 - 80.3 (FIG. 2). The line through the experimental points was obtained by least-squares fit and the correlation coefficient is 0.99. In the range of ϵ_r 4.7 - 2.6 the deviations from linearity to superlinearity are detected and this can be interpreted as some specific solvent-solute interactions /1/. Ascertained linear dependence λ_A vs ϵ_r in the range of ϵ_r 4.8 - 80.3 enhances the applicability of M-540 as a polarity probe, also in biological samples or models. In this work we present visible electronic spectra of M-540 in model phospholipid membranes - liposomes (FIG. 3). These spectral data are summarized in TAB. 1. As can be seen from FIG. 3 and TAB. 1, M-540 in different lecithins exhibits only slight spectral shifts of 2.6 nm (0.009 eV) while passing from OPL to DPL liposomes. Our data of

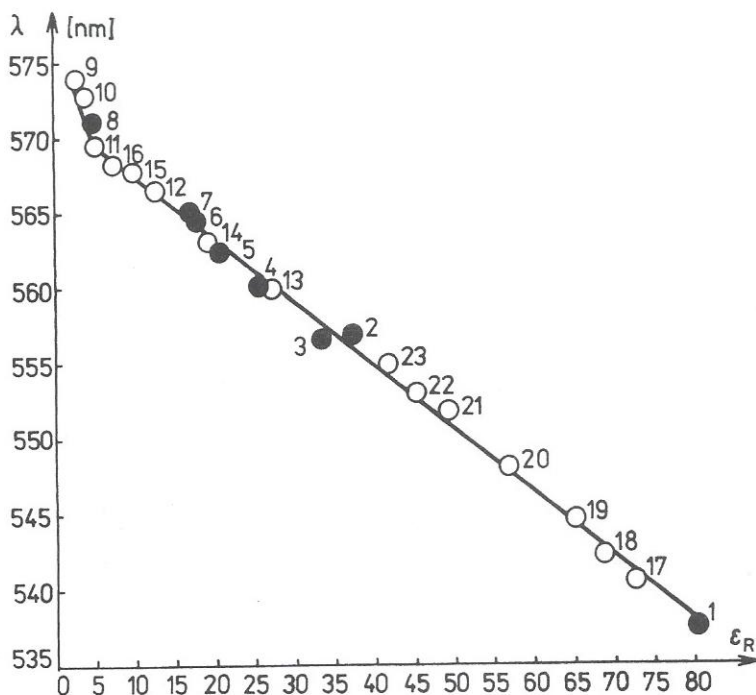


FIG. 2 Plot of the absorption maximum λ_A of A peak of M-540 vs the solvent polarity parameter ϵ_r . For $\epsilon_r \in <4.8; 80.3>$ the linear correlation is given:
 $\lambda_A = (571.7 \pm 1.5) - (0.42 \pm 0.03) \times \epsilon_r$.

λ_A of M-540 in EL and DPL liposomes can be compared with the data of M-540 in phospholipid vesicles [10]. We observed a slightly different λ_A shifted to longer wavelengths (3 - 5 nm) which can be attributed to different sample preparation (different temperature, different pH of water and presence of KCl in water). From the data in TAB. 1 and the calibration curve in the FIG. 2, the effective values of the dielectric constant ϵ_r of the dye binding environment in lecithin (EL, OPL, DML, DPL) liposomes can be only roughly estimated within the interval 3 - 6. The more precise value is not realistic as the values have been taken from FIG. 2 between 569,5 nm and 572,1 nm where the curve is no longer linear. Furthermore the wavelength difference of only 3 nm seems to be itself within the limits of error. The obtained spectral characteristics of the M-540 probe will be utilized for further investigation of membrane structure and dynamics by the fluorescent probe M-540.

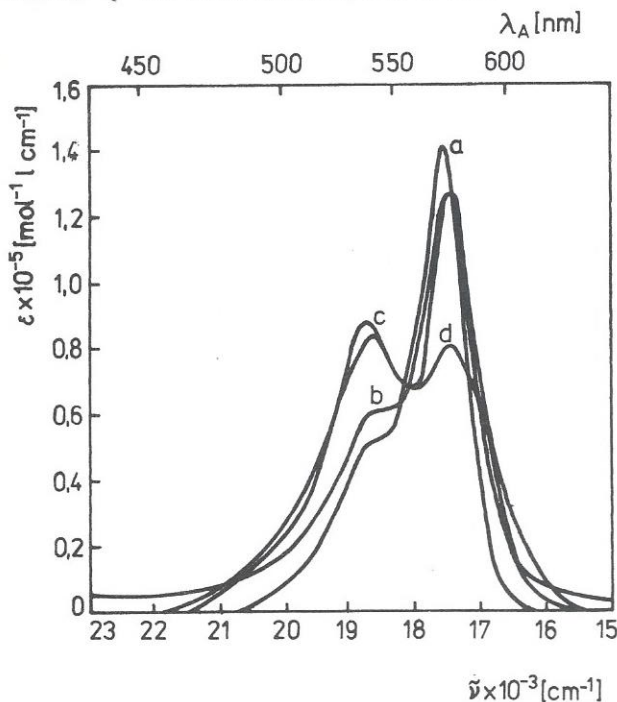


FIG. 3 Absorption spectra of M-540 in liposomes of a) OPL, b) EL, c) DML, d) DPL (concentrations of M-540 $c = 3 \times 10^{-5}$ mol/l).

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В работе были измерены спектры поглощения мероцианина 540 (M-540) в различных растворителях. При этом мы наблюдали гипсохромный сдвиг максимума поглощения красителя. Для количественной оценки сольватохромизма зонда M-540 мы построили калибрационную зависимость положения максимума спектра поглощения от диэлектрической проницаемости, характеризующей полярность растворителя. Полученные спектральные характеристики позволили оценить полярность окружения зонда в искусственных фосфолипидных мембранах.

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