

*Dedicated to Professor Ioan Bâldea on the
Occasion of His 80th Anniversary*

OPTICAL PROPERTIES MODULATION OF CYANINE DYES IN ORGANIC SOLVENTS AND IN THE CRITICAL INTRACELLULAR pH WINDOW

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ABSTRACT. The synthesis of new 2-(2-(3-formyl-10*H*-phenothiazin-7yl)vinyl)-3*H*-indolium iodide and comparison of the UV–visible absorption/emission spectral changes for several indolium cyanine dyes upon variation of organic solvents and aqueous solutions with pH in the range between 5–8 pH units were described.

Keywords: cyanine dyes, phenothiazine, indolium iodide, solvatochromism, pH sensitivity

INTRODUCTION

In a complex biological material, a pH-sensitive dye can act as a pH-dependent reporter molecule which can be conveniently spotted by using non-invasive spectroscopic absorption/emission detection in the near-infrared region of the electromagnetic spectrum ($\lambda_{\max} > 650$ nm), where the biological medium is extensively penetrated; thus, labeling of biological macromolecules with a near-infrared chromophore became a documented approach in bioanalytical research [1]

Cyanine dyes is a class of functional dyes of greet interest in many fields due to their high extinction coefficients and tunable optical properties. Trimethine cyanine dyes containing indolium units were reported as displaying observable changes of fluorescent emission within the critical intracellular pH

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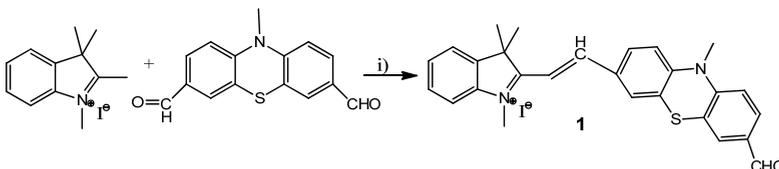
range (6–8) [2]. A near-infrared, pH sensitive colorimetric fluorescent probe containing the dihydro-xanthenes-indolium dimethine cyanine chromofore offered great potential for in vivo bioanalytical applications such as palladium detection and bioimaging [3]

The UV-Vis absorption/emission properties of several dimethinecyanine dyes containing phenothiazine as the core donor and indolium salt as the acceptor previously described, pointed out large bathochromic shifts of the absorption band in halogenated solvents [4] and large Stokes shifts of fluorescence emission, indicative of a great influence of the geometry of the emissive excited state [5]

In this work, we report the synthesis of a new dimethine cyanine dye containing a formyl substituted phenothiazine unit and indolium salt as the acceptor. A comparison of the UV-Vis absorption/emission spectral properties of di-, penta- and heptamethine cyanine dyes containing indolium acceptor moiety, recorded in solvents of different polarity as well as in aqueous buffer solutions simulating the critical intracellular pH window of 5–8 pH units, is reported.

RESULTS AND DISCUSSION

The synthesis of new 2-(2-(3-formyl-10*H*-phenothiazin-7yl)vinyl)-3*H*-indolium iodide **1** was performed by Knoevenagel condensation of 1,2,3,3-tetramethyl-3*H*-indolium iodide with 10-methyl-10*H*-phenothiazine-3,7-dicarbaldehyde as presented in scheme 1.



Scheme 1. Synthesis of cyanine dye **1**. i) piperidine, ethanol, 12 h, 70 °C.

The structural assignments of **1** were based on spectroscopic data. The ¹H-NMR spectrum displayed the key signals generated by the protons belonging to the vinyl bridge as doublets with vicinal coupling constants of 16.4 Hz pointing the formation of the geometrical *trans*- isomer, while the signal of the CH=O proton was situated downfield at 9.8 ppm.

The electronic absorption and spectra of **1** recorded in different solvents are presented in Figure 1 exhibiting a solvatochromism of the absorption maxima positioned in the visible range (542-592 nm).

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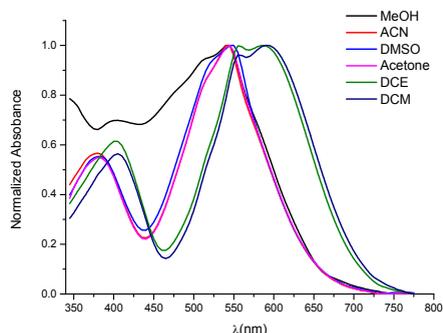


Figure 1. Normalized UV/visible absorption spectra of **1** in various solvents.

Optical properties modulation of cyanine dyes

Several cyanine dyes: chloroheptamethine **2**, cyanine containing the dihydroxanthene heterocyclic unit **3** and bromopentamethine **4** (figure 2) (obtained according to previously reported procedures) were employed in a comparative study of optical properties modulation in the presence of organic solvents of different polarity and aqueous solutions of variable pH, respectively.

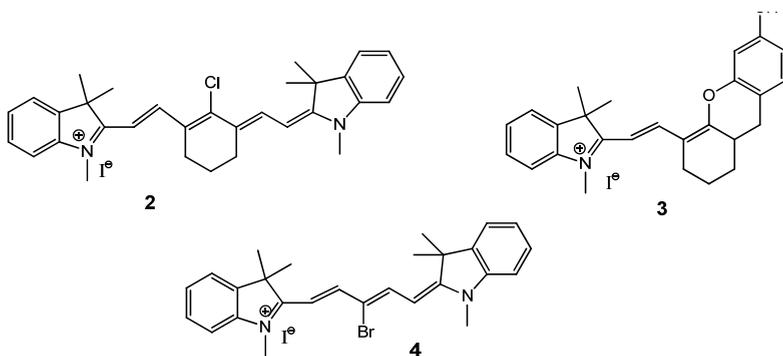


Figure 2. Structure of cyanine dyes employed in the optical properties study

UV-Vis absorption spectra in organic solvents

In figure 3 are displayed the UV-absorption spectra of the cyanine dyes **1-4**, depicting a red shift of the longest wave absorption maxima directly correlated to the length of the pi conjugated system between the donor moiety and the acceptor indolium core.

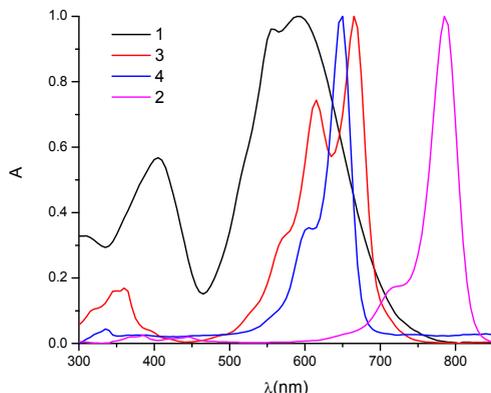


Figure 3. Normalized UV-absorption spectra of the cyanines **1-4**

In Table 1 are summarized the positions of the characteristic absorption maxima recorded in various solvents. A bathochromic shift of the absorption maxima can be observed for each cyanine dye when halogenated solvent dichloromethane was used as a solvent. This behavior was also reported for other dyes with donor– π –acceptor structure and it was rationalized based on halogen–halide interaction responsible for raising the ground state energy level [6]. The hypsochromic shift observed upon increasing solvent polarity can be explained by a better stabilization of the ground state which appears more polar than the excited state. [5]

Table 1. Solvatochromism of UV-Vis longest wave absorption maxima for cyanine dyes **1-4**

Solvent	1	2	3	4
	$\lambda_{\max}(\text{nm})$	$\lambda_{\max}(\text{nm})$	$\lambda_{\max}(\text{nm})$	$\lambda_{\max}(\text{nm})$
Methanol	542	770	657	633
Acetonitrile	542	771	649	635
Acetone	542	774	655	635
Dimethylsulfoxide	545	784	665	644
Dichloromethane	592	784	665	649

Fluorescence emission spectra in organic solvents.

Upon irradiation with the longest wave absorption maxima, cyanines **2-4** displayed fluorescence emission characterized by larger Stokes shifts in dimethyl sulfoxide solvent as compared to dichloromethane solvent (table 2), pointing towards a better stabilization of the excited state by polar solvent interactions. For cyanine **1** was not observed any fluorescence emission in solution.

Table 2. Fluorescence emission maxima of cyanine **2-4** in organic solvents

Solvent	2 λ_{em} (nm)	3 λ_{em} (nm)	4 λ_{em} (nm)
Dimethylsulfoxide	812	747	656
Dichloromethane	803	685	654

UV-Vis absorption/emission properties in aqueous solutions of variable pH

The UV-Vis absorption/emission properties of pH sensitive cyanine dyes **2-4** were studied in aqueous buffer solutions of pH 5, 6, 7, and 8 units respectively. In the case of **4** lower pH-values directed the protonation of the tertiary heterocyclic nitrogen atom in the indole free base donor core, determining the hypochrome effect upon absorption and emissions. In figure 4 are presented the UV/visible absorption/emission spectra of cyanine **4** as a function of pH. For the cyanine **3**, solution of higher pH generated the fenoxide auxochrome group which determined a bathochromic shift of the absorption and emission maxima. Table 3 summarizes the shifts of the UV-Vis absorption/emission maxima upon modulation of pH in aqueous solutions.

Table 3. The UV-Vis absorption/emission properties of pH sensitive cyanine dyes **2-4**

pH	2		3		4	
	$\lambda_{max}(nm)$	λ_{em} (nm)	$\lambda_{max}(nm)$	λ_{em} (nm)	$\lambda_{max}(nm)$	λ_{em} (nm)
5	769	792	649	672	633	650
6	769	778	649	671	633	650
7	769	778	681	704	633	650
8	769	778	681		633	650

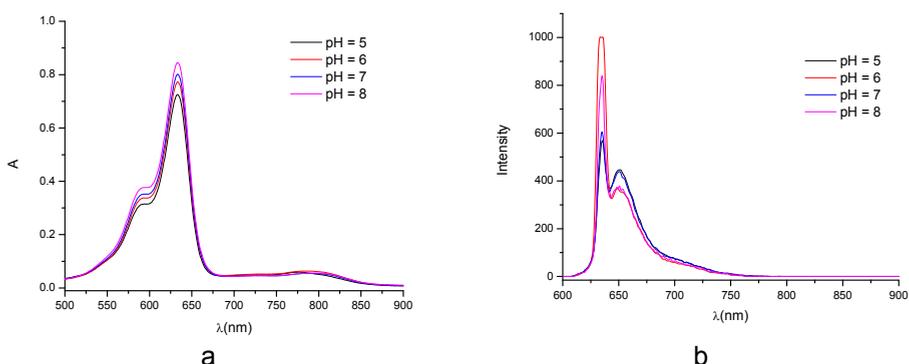


Figure 4. UV-Vis spectra of **4** recorded in aqueous buffer solutions of variable pH
a) absorption ($C = 10^{-5}$ M) b) emission ($C = 10^{-5}$ M)

CONCLUSIONS

The optical properties of the cyanine dyes containing indole units can be slightly modulated by using organic solvents of different polarities as well as aqueous solutions of different pH.

EXPERIMENTAL SECTION

Materials and Methods

The starting materials and solvents were obtained from commercial sources from Sigma-Aldrich. NMR spectra were recorded at room temperature on 400 MHz Bruker Avance instruments. Chemical shifts are expressed in δ (ppm) relative to standard tetramethylsilane (TMS).

Compounds **2-4** were prepared according to the previously reported procedure: **2** [1], **3** [3.], **4** [7].

2-(2-(3-formyl-10H-phenothiazin-7yl)vinyl)-3H-indolium iodide 1

10-methyl-3,7-diformyl-phenothiazine (0,4 g, 1,5 mmol) and 1,2,3,3-tetramethyl-indolium iodide were mixed in a Schlenk flask. Then 20 ml of ethanol and catalytic amount of piperidine (100 μ l) was added to the mixture under inert atmosphere. The obtained solution was stirred for 12 hours at 70°C. The reaction progress was monitored by thin layer chromatography (TLC, eluent DCM/EtOH = 80:1). After the completion of the reaction the solid product was isolated from the reaction mixture by vacuum filtration. The crude product was then purified using column chromatography (solid phase: silica gel, eluent: gradient solution from DCM/EtOH = 80:1 to DCM/EtOH = 2:1). The pure product was obtained in 55% yield.

MS (*m/z*, *EI*, 70eV): 425 (M^+)

¹H-NMR (400 MHz, DMSO-*d*₆): δ_{ppm} = 1,77 (s, 6H, 2·CH₃), 3,5 (s, 3H, PtzN-CH₃), 4,12 (s, 3H, N-CH₃), 7,19-7,24 (m, 2H, H₁, H₉), 7,54-7,63 (m, 3H, H_a, H₈, H_{Ar}), 7,69 (d, 1H, H₆), 7,79 (dd, 1H, H_{Ar}), 7,85-7,87 (m, 2H, H_{Ar}), 8,09 (d, 1H, H_{Ar}), 8,15 (d, 1H, H_{Ar}), 8,33 (d, 1H, H_b, ³*J*=16,44 Hz), 9,85 (s, 1H, CHO);

¹³C-NMR (100 MHz, DMSO-*d*₆): δ_{ppm} = 25,9; 34,7; 36,8; 52,3; 111,6; 115,4; 116,2; 116,3; 122,2; 123,3; 127,8; 128,1; 129,3; 129,5; 130,4; 131,2; 132,2; 133; 142,3; 143,9; 148,4; 149; 152; 181,7; 191,3;

Buffer solutions

Sodium acetate and sodium phosphate buffers were prepared, at three different pH 5.0, 6.0 and 7.0, respectively. The pH of sodium acetate buffer was adjusted with acetic acid or sodium hydroxide solution. The sodium phosphate buffer was prepared by mixing different volumes of 1 M NaH_2PO_4 and 1 M Na_2HPO_4 stock solutions to obtain three different pH values: 5.0, 6.0 and 7.0, respectively. Phosphoric acid was used to adjust pH to 5.0.

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REFERENCES

1. L. Strekowski, J.C. Mason, H. Lee, M. Say, G. Patonay, *J. Heterocyclic Chem.*, **2004**, *41*, 227].
2. R.M. El-Shishtawy, P. Almeida, *Tetrahedron*, **2006**, *62*, 7793.
3. Su W., Gu B., Hu X., Duan X., Zhang Y., Li H., Yao S., *Dyes and Pigments*, **2017**, *137*, 293.
4. B. Brém, Q. Colange, E. Gal, D. Porumb, C. Cristea, L. Găină, T. Lovász, L. Silaghi-Dumitrescu, *Studia UBB Chemia*, **2018**, *63(2)*, 117.
5. R.M. El-Shishtawy, F.A.M. Al-Zahrani, S.M. Afzal, M.A.N. Razvi, ZM. Alamshany, A.H. Bakry, AM. Asiria, *RSC Advances*, **2016**, *6*, 91546.
6. Y. Ooyama, Y. Oda, T. Mizumo and J. Ohshita, *Tetrahedron*, **2013**, *69*, 1755.
7. Hu H., Owens E. A., Su H., Yan L., Levitz A., Zhao X., Henary M., Zheng Y. G., *J. Med. Chem.*, **2015**, *58(3)*, 1228.

