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SPECTRAL PROPERTIES OF A *bis*-AZOSPIROPYRAN COMPLEXED WITH EUROPIUM

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The complexation of recently synthesized symmetrical bifunctional bis-azospiropyran photochromic dye with europium nitrate and its effect on UV-Vis absorption and fluorescent emission was studied. Upon addition of Eu^{3+} *to colorless spiropyran, a yellow merocyanine europium complex was obtained with an absorption band at 410 nm. Negatively charged phenolic oxygenin zwitterionic ring-open form provides an effective metal binding site for* Eu^{3+}*. Meanwhile, the inherent fluorescence emission of the photochromic dye at 380 nm is switched off due to the* Eu^{3+}*-induced drive of spiro-mero equilibrium to form mero form. The stoichiometry of dye–europium complexation was evaluated by fluorescence emission and UV-Vis absorption spectroscopy and a 8:1 ratio was obtained in both cases. The binding constant (K) value of the dye–europium complex was* $3 \times 10^6\ M^{-1}$*. In conclusion, the current molecular switch is a useful sensitive dual measuring tool for solutions containing europium or europium-like elements by evaluation of visible absorption or fluorescent emission spectroscopy.*

Keywords: *lanthanides, photoswitch, complexation, photoresponsivity, azospiropyran.*

Introduction. Lanthanides have found numerous applications in the ceramic and glass industry, metallurgy, electronics, agriculture, and natural sciences. Their ionic forms are also becoming very important in different industries. Europium (Eu), as a member of lanthanides, is found to have many applications as a fluorescent agent in anodic rays of television and monitor screens [1–8]. Also, europium nitrates are frequently singled out for attention due to their significant role in signal enhancement of antibody microarrays in most sensitive and reliable immunoassay platforms [9].

The available methods for low-level determination of Eu(III) ions in solutions are spectroscopy, mass spectrometry (MS), inductively coupled plasma atomic emission spectrometry (ICP-AES) and inductively coupled plasma mass spectrometry (ICP-MS), isotope dilution mass spectrometry, neutron activation analysis, and X-ray fluorescence spectrometry [10–13]. Chemosensory systems based on receptor–guest complexation for specific chemicals promise a nondestructive and convenient method of analysis for biologically and environmentally important specimens. To date, a number of synthetic receptors integrated with chromophores and fluorophores have been reported for colorimetric and fluorometric sensing, respectively [14].

Spiropyrans, as well-known photochromic compounds [15, 16], are usually colorless in their ring close spiro (Sp) forms, but their ring-open merocyanine (Mc) forms produce different colors upon exposure to UV light. It is also reported that their SP–Mc isomerization in the presence of metal ions is possible under dark conditions [17]. Among the spiropyrans, only a few reports dealt with the synthesis of *bis*-spiropyrans [17, 18]. However, the development of such *bis*-spiropyrans has led to the invention of highly sensitive molecular switches with bifunctional chromophores in one molecule. In comparison with mono-spiropyran, higher sensitivity and molar capacity was observed in the presence of two zwitterionic merocyanine units [18]. Moreover, to best of our knowledge, the first report on the synthesis of *bis*-azospiropyran systems has recently been published by the authors [19]. In keeping with the line of research on synthesis and properties of *bis*-azospiropyrans dyes [15, 19] this is a report of europium nitrate complexation with a highly sensitive photochromic *bis*-azospiropyran (BASP) dye, 3,3'-[benzene-1,4-diylidi(E)diazene-2,1-diyl]*bis*[1',3',3'-trimethylspiro(2H-1-benzopyran-2,2'-indolin)-6-yl] (**1**) and its effect on UV-Vis absorption and fluorescent emission.

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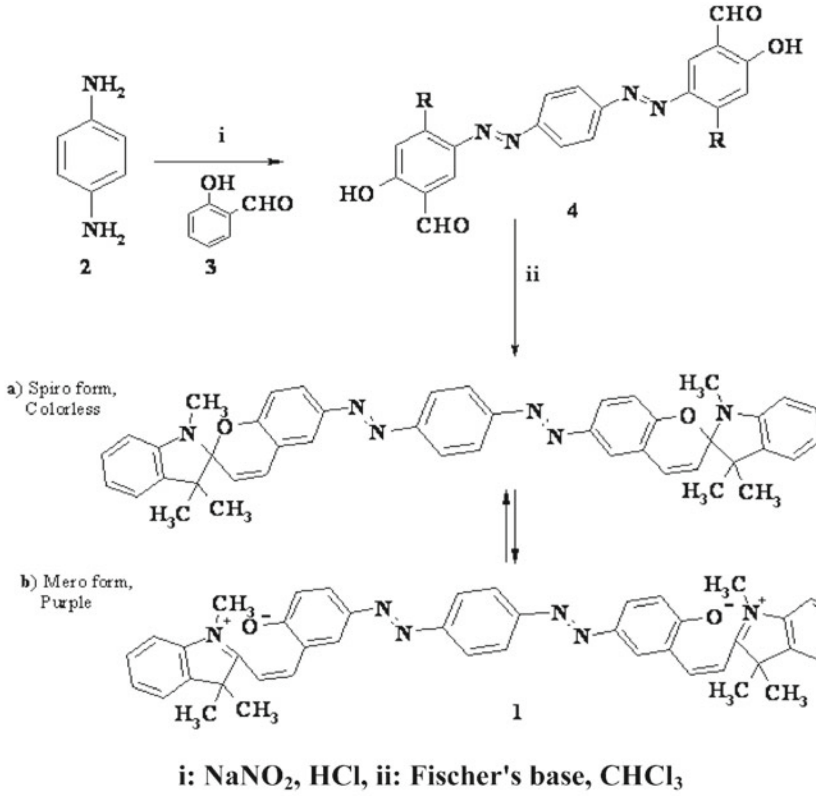
Experimental. All chemicals of analytical grade were purchased from well-known chemical companies such as Sigma-Aldrich and Merck chemicals and used as received. Double distilled deionized water was used throughout. $Eu(NO_3)_3$ stock solution was prepared from Eu_2O_3 (323543 ALDRICH, 99.999%) after dissolving a certain amount of Eu_2O_3 in hot diluted nitric acid. Finally, a transparent solution was formed [20]. UV-Vis spectra were recorded using a Multispec-1501 Shimadzu UV-Vis spectrophotometer. Fluorescence spectra were measured using a Perkin Elmer LS 55 fluorescence spectrometer.

The symmetric *bis*-azospiropyran dye **1** was synthesized according to our previous report [19]. Briefly, a solution of $NaNO_2$ (1.6 g, 23.2 mmol) in H_2O (5 ml) was added to salicylaldehyde **3** (2.66 g, 21.8 mmol) in H_2O (10 ml) including NaOH (1.8 g) at 5°C. The resulting solution was added dropwise to the solution of *p*-phenylenediamine **2** (1.25 g, 11.6 mmol) in HCl (10 ml, 1%) at 0°C. The mixture was stirred for 1 h at room temperature; then the precipitate was filtered and washed out with distilled water. Compound **4** was crystallized in EtOH to afford pure **4**.

2-Methylene-1,3,3-trimethylindoline (0.352 ml, 2 mmol) in 5 ml $CHCl_3$ was added dropwise to a refluxing solution of **4** (1 mmol) in 50 ml $CHCl_3$, in 20 min. The mixture was refluxed for 2 h and then cooled to room temperature. Then the precipitate was filtered, washed with distilled water, and crystallized in EtOH to afford the pure spiro compounds **1**.

3,3'-[Benzene-1,4-diylidi(E)diazene-2,1-diyl]*bis*[1',3',3'-trimethylspiro(2H-1-benzopyran-2,2'-indolin)-6-yl] (**1**) Yield (89%). White powder. M.p. 203–205°C. IR (KBr): 2966 (CH=), 1605 (C=C), 1311 (N–N), 1021 (C–O). ^1H NMR (DMSO-d_6): 1.25 (s, 2 Me); 1.28 (s, 2 Me); 2.80 (s, 2 NMe); 4.20 (d, $^3J_{HH}$ =10.2, 2 H–C(3)); 6.60–7.26 (m, 18 arom. H, and 2H–C(4)). MS *m*/*z* (%) = 685 (*M*+1, 1), 655 (*M*–2 Me, 1), 493 (15), 404 (2), 318 (88), 274 (25), 212 (100), 183 (18). Anal. calc. for $C_{44}H_{40}N_6O_2$ (684.84): C 77.17, H 5.89, N 12.27; found: C 77.20, H 5.85, N 12.30.



Scheme 1. Synthesis pathway of the symmetric *bis*-azospiropyran dye **1**, colorless spiro (a) to merocyanine (b) forms.

Results and Discussion. *Complexation of BASP with europium ion (Eu^{3+}).* As we reported earlier [15, 19], BASP changes from colorless spiro (Sp) to merocyanine (Mc) form upon exposure to 366 nm UV light (Scheme 1). The maximum color of the produced Mc-form was obtained after exposure to UV light for less than 2 min at ambient temperature (Fig. 1a). The molar absorption coefficient of an unsubstituted spiropyran in its Mc form was 0.31×10^4 l/M · cm; which increased to

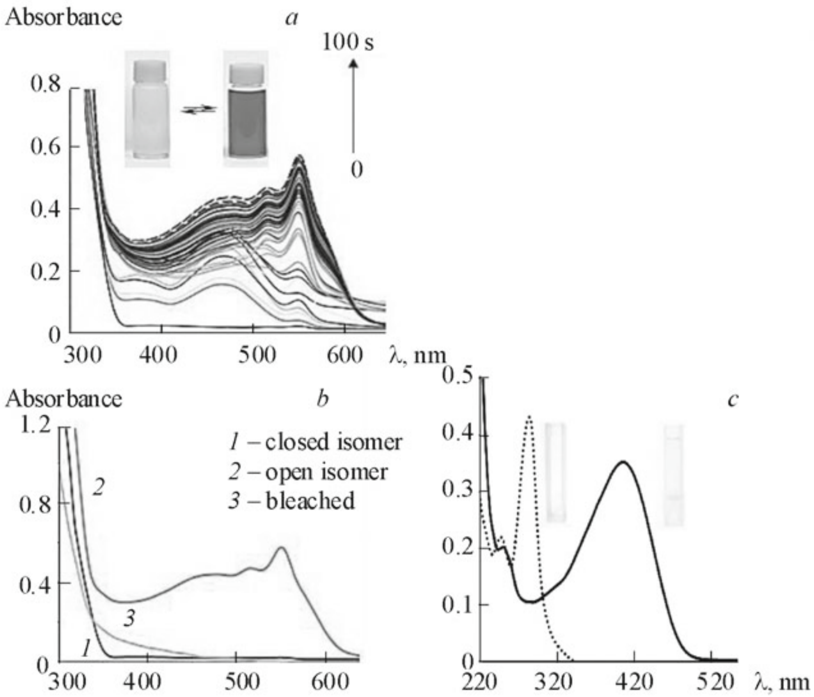


Fig. 1. UV-Vis absorption spectra of BASP **1** ($C = 2 \times 10^{-4}$ mol/l in CH_2Cl_2) during 100 s exposure to 366 nm UV light (a); the reversibility of mero to spiro forms at 122°C (b), absorption spectra of BASP (1×10^{-5} M, CH_3CN, 293 K) with and without $Eu(NO_3)_3$ (0.125 equiv) (c).

1.35×10^4 l/M · cm in the presence of an azo moiety in the Mc form of monoazospiropyran. It is well known that sensitivity and resolution of discrimination between ON (Mc) and OFF (Sp) forms in the molecular switch depend on molar absorption coefficient and the higher molar absorption coefficient will mean better discrimination. The molar absorption coefficient of the constructed *bis*-azospiropyrans was 3.8×10^4 l/M · cm [15, 19] which is astonishingly high. So, *bis*-azospiropyrans have higher discriminative power than monoazospiropyran or unsubstituted spiropyran.

As expected, the presence of negatively charged phenolic oxygen in the zwitterionic open form and the resulted phenolate anion act as a chelating site for effective metal binding. Hence, the addition of aqueous solution of Eu^{3+} to the colorless (Sp) form of dye **1** in acetonitrile in the dark causes a color change of the solution from colorless to deep-yellow (Fig. 2a). Accordingly, absorption spectral changes of BASP (1×10^{-5} M, CH_3CN, 293 K) were studied after addition of 0.125 equivalent of $Eu(NO_3)_3$ and revealed a bathochromic shift of 125 nm in *the maximum absorbance wavelength* from 285 to 410 nm (from UV to visible wavelengths) (Fig. 1c). The absorptions were measured after overnight incubation to ensure stabilization of the equilibrium [21].

Binding studies with absorption of visible light. The complexation stoichiometry of Eu with Mc was investigated by evaluating the absorbance at 410 nm and the concentration of metal salt. The absorbance at 410 nm reached a plateau after addition of an equivalent amount of Eu^{3+} to the solution of BASP (Fig. 2b). As shown in the figure, the apex is located at an europium molar ratio of 0.125, which is consistent with a Mc–Eu stoichiometry of 8:1. An isomolar solution technique or Job's method of continuous variations (Fig. 2c), which has been successfully applied in the analysis of other spirochromene metal complexes [21, 22], was also used to double check the obtained stoichiometry, and the same result, i.e., 8:1 ratio, was obtained (Fig. 2d).

The binding constant (K) value of the Mc–Eu complexation was determined using the absorbance intensities recorded at different Eu concentrations and the modified Benesi–Hildebrand equation

$$1/\Delta A = 1/\Delta A_{\max} + (1/K[C])(1/\Delta A_{\max}) , \tag{1}$$

$$\Delta A = A_x - A_0, \Delta A_{\max} = A_\infty - A_0 ,$$

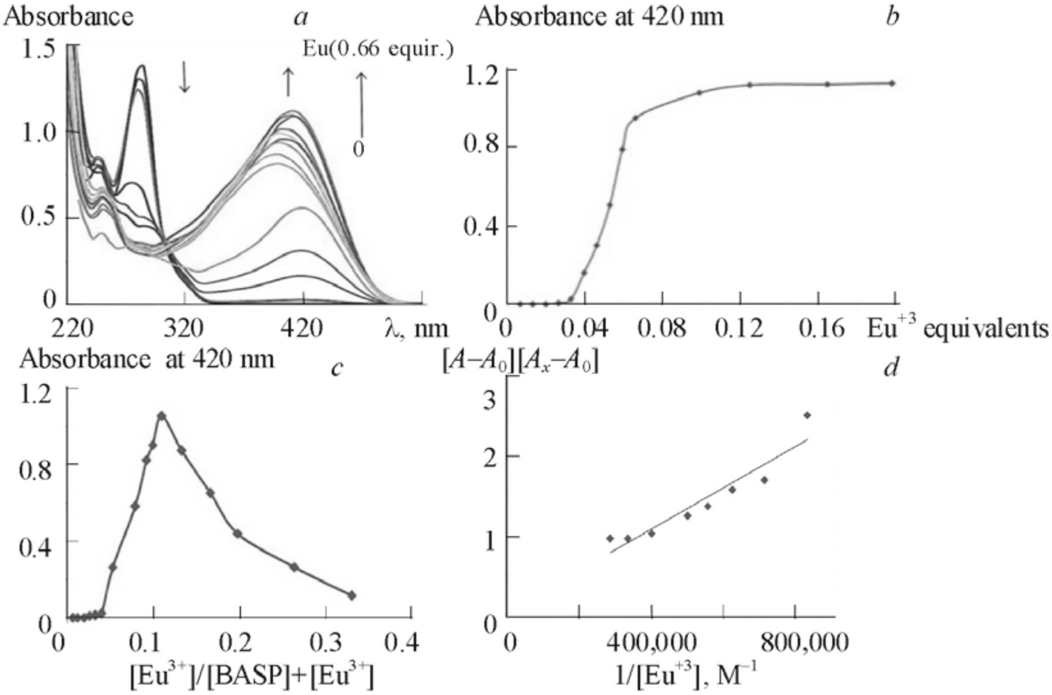


Fig. 2. Absorption spectra of a BASP solution (5×10^{-5} M, CH_3CN, 293 K) after addition of 0.0 to 0.66 equivalents of $Eu(NO_3)_3$ for a night (a); absorption at 410 nm of a solution of BASP (3×10^{-5} M, CH_3CN, 293 K) after increasing the concentration of $Eu(NO_3)_3$ (b); Job's analysis of the Mc–Eu complex ([BASP] + [Eu^{+3}] = 3×10^{-5} M, CH_3CN, 293 K) (c); plot of $(A_\infty - A_x)/(A_x - A_0)$ versus 1/[Mc–Eu] (d).

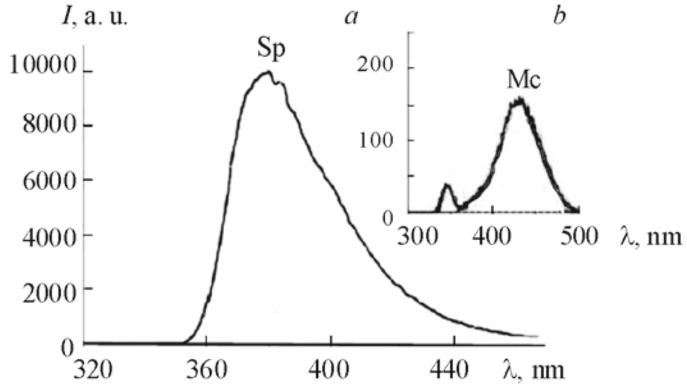


Fig. 3. Fluorescence emission spectra of BASP **1** in Sp (a) and Mc (b) forms in DMF ($C = 2 \times 10^{-4}$ mol/l).

where A_0, A_x, and A_∞ are the absorption intensities of the Mc–Eu complex at 410 nm in the absence of metal, a certain concentration of metal, and the concentration of a complete interaction, respectively; K is the binding constant, and $[C]$ is the concentration of metal as shown in Fig. 2. The K value of the Mc–Eu complex was estimated by extrapolating the slope of $(A_\infty - A_0)/(A_x - A_0)$ versus 1/[Eu^{3+}], which showed a value of 3×10^6 M^{-1}.

Binding studies with fluorescent emission of the complex. Fluorescent emission at 380 nm was obtained for the Sp form of the BASP compound 1 (2×10^{-4} mol/l in DMF) (Fig. 3a). It faded out due to Mc formation after irradiation with UV light, and a new low intensity emission at 440 nm was observed (Fig. 3b). The UV light induced Mc formation and fading of the Sp form due to fluorescent emission of BASP was reported earlier [19].

In the presence of Eu^{3+}, emission of the Sp form of BASP faded out and a new low intensity emission of the Mc–Eu complex was observed at 526 nm, indicating an 86 nm red shift. Such findings might have resulted from the presence of a

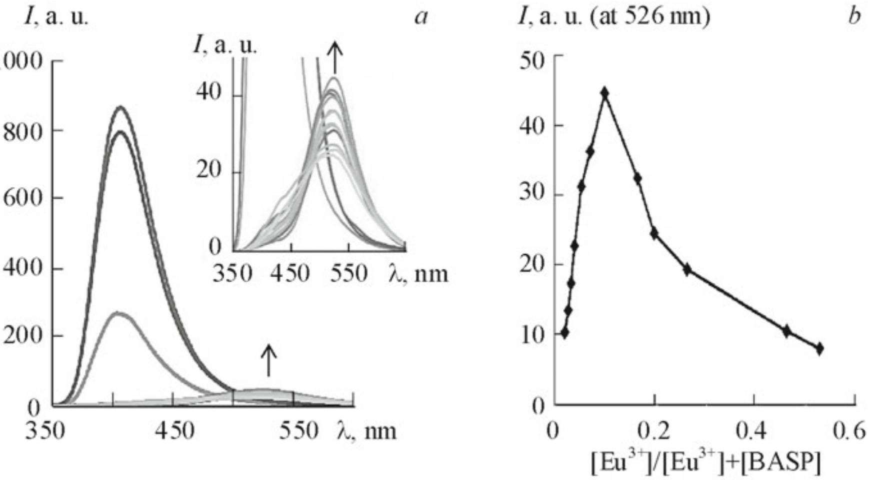


Fig. 4. Emission spectra of a solution of BASP (3×10^{-5} M, CH_3CN, 293 K) after one night from the addition of 0.0 to 0.66 equivalents $Eu(NO_3)_3$ (a); Job's analysis of the Mc–Eu complex ([BASP] + $[Eu^{+3}]$ = 3×10^{-5} M, 293 K) (b).

zwitterionic open (Mc) form with an effective binding site for the added Eu^{3+}. In fact, the addition of Eu^{3+} to BASP favors an equilibrium between the Sp and Mc forms for formation of a ring open (Mc) form, and it switches off the Sp related fluorescence emission at 380 nm and switches on the Mc–Eu complex related emission at 526 nm (Fig. 4a). The emission spectra of a BASP solution (3×10^{-5} M, CH_3CN, 293 K) after overnight incubation with different equivalents of $Eu(NO_3)_3$ (0.0, 0.07, 0.013, 0.02, 0.03, 0.033, 0.04, 0.05, 0.053, 0.06, 0.07, 0.10, 0.13, 0.17, 0.20, 0.264, 0.33, 0.66) are illustrated in Fig. 4a.

Based on the aforementioned results (Fig. 4a), the Mc–Eu complex-related emission at 526 nm was chosen for all subsequent binding studies. The complexation ratio was calculated by computer fitting the emission/molar ratio data. The stoichiometry and stability constants for the complex in the presence of varying amounts of Eu^{3+} were studied by analysis of emission spectra. The formation constant of the resulted complex was also evaluated by Job's method of continuous variations as discussed before (Fig. 4b). As it is shown in Fig. 4a, the experimental data reveal a very good correlation, indicating the formation of 8:1 Mc–Eu complex with an equilibrium constant of 3×10^6.

Conclusion. Here, we report a highly sensitive complexation between europium nitrate and photochromic *bis*-azospiropyran (BASP) dye, 3,3'-[benzene-1,4-diylidi(E)diazene-2,1-diyl]*bis*[1',3',3'-trimethylspiro(2H-1-benzopyran-2,2'-indolin)-6-yl], which forms an 8:1 complex**,** with an equilibrium constant of $(3 \pm 0.02) \times 10^6\ M^{-1}$. Due to the characteristics of novel *bis*(azospiropyran) BASP and its complexation with europium nitrate, the process could be monitored via both UV-Vis absorption and fluorescent emission spectroscopy. Thus, the current molecular switch is a useful dual measuring tool for solutions containing europium or europium-like elements that can be examined by color changes or a red shift of the fluorescent emission wavelength.

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