Available online at www.sciencedirect.com

Dyes and Pigments 62 (2004) 213–219

www.elsevier.com/locate/dyepig

The effect of the microenvironment on spectroscopic properties of complexes comprised of stilbazolium merocyanine and Cu(II) and Co(II) ions in isotropic and anisotropic media

Roman Cegielski*

Faculty of Physics, Poznań University of Technology, Nieszawska 13 a, 60-965 Poznań, Poland

Received 29 July 2003; received in revised form 21 November 2003; accepted 2 December 2003

Abstract

The effect of the microenvironment on the spectroscopic properties of the complexes comprised of the derivative of merocyanine and specific ions (either dissolved or embedded in the polymer matrix) was examined. The effect of water concentration and the concentration of various anions on complex-forming ability was also established in this paper. As shown here, the mero–Co^{2+} complex undergoes dissociation in the presence of water. However, for mero–Cu^{2+} complex only a hipsochromic shift in the absorption spectrum was observed. The presence of the examined anions exhibited a strong effect on the formation of the mero–Cu^{2+} complex. In anisotropic polymer matrix, the examined complexes also exhibited discrepant features.

Keywords: Stilbazolium merocyanine; Transition metal ions; Absorption; Complexes

1. Introduction

Copper and cobalt ions are known to be indispensable for allowing every living organism to perform its normal functions. However, an excess of these ions can create a serious threat [1]. As was concluded from earlier research, both ions are able to catalyze and distinctly advance various cellular reactions. These reactions are responsible for the formation of an abundance of extremely reactive oxygen species which can generate irreversible damage to numerous intracellular structures [2,3].

In a time of dynamic industrialization and rapid development of the chemistry of materials, the ions of cobalt and copper (both soluble in water) constitute a substantial element of environmental pollution. All efforts to develop an effective procedure to remove these ions from industrial waste are crucial [4]. However, the capacity for monitoring the migration and the concentration of these ions in the various links of a food chain is equally critical. As a consequence, the selection of the most effective tool allowing the detection of the presence of Cu^{2+} and Co^{2+} ions in both the

---

* Tel.: +48-61-665-31-86; fax: +48-61-665-32-01.
E-mail address: cegielsk@phys.put.poznan.pl (R. Cegielski).

0143-7208/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.dyepig.2003.12.005

environment and in the cells of the living organisms has become an urgent and unavoidable necessity.

The electron spectra of numerous derivatives of stilbazolium betaines display very high sensitivity to even subtle deviations in the microenvironment [5]. Therefore, they create a large group of compounds with a high potential of serving as such tools. Moreover, these dyes demonstrate a significantly high affinity to the cellular membranes of both human adenocarcinoma cells and human peripheral blood leukocytes [6–8].

Based on previous observations [9,10], a singular derivative of stilbazolium merocyanine, namely the one carrying the hydroxyl group in position ortho in relation to the carboxyl group of the quinonoid ring, is viewed as particularly hopeful. In the presence of Cu^{2+} and Co^{2+} ions, this compound demonstrated measurable modifications in its visible light absorption spectra and its EPR spectra. The appearance of a new long wave band was generated in the presence of both Cu^{2+} and Co^{2+} ions. The maximum of this band was located at different wavelengths specific to the respective ion.

The research presented in this paper was motivated by two major goals. Firstly, to establish (by using the UV–Vis absorption spectroscopy) if a presence of various anions in a microenvironment of the merocyanine has any measurable effect on its ability to form complexes with Cu^{2+} or Co^{2+} ions. Secondly, to establish the behavior of formed complexes in anisotropic polymeric matrix serving as a model for biological cellular membrane.

2. Materials and methods

2.1. Chemicals and procedures

1-(12-Hydroxydodecyl)-4-[(3-hydroxy-4-oxocyclohexa-2,5-dienylidene)-ethylidene]-1,4-dihydropyridine was used as a dye. Its structure is shown in Fig. 1. The procedure for the synthesis of merocyanine was described earlier [11]. Melting point, IR and NMR spectra were presented previously [8]. In the research presented in this paper, fallowing salts of transition metals were used: $Cu(CH_3COO)_2$, $Co(CH_3COO)_2 \times 5H_2O$, $CuSO_4 \times 5H_2O$, $CuCl_2 \times 2H_2O$ and $CoCl_2 \times 6H_2O$. These salts were purchased from Fluka, Aldrich, and POCh-Lublin. The polyvinyl alcohol (PVA) was purchased from Sigma. The polyvinyl alcohol film was prepared according to the procedure described previously [12]. The pH of each measured sample was controlled.

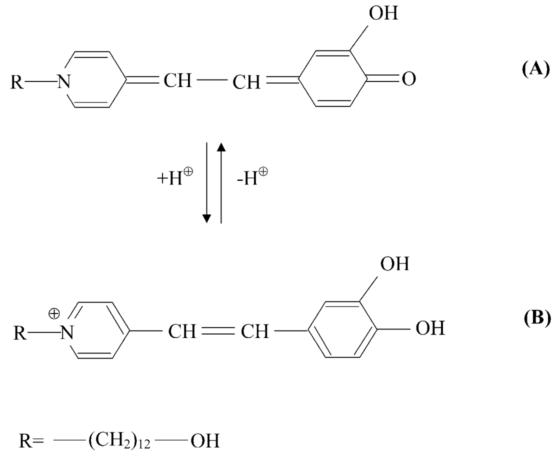

Fig. 1. The structure of the basic (A) and protonated (B) forms of tested merocyanine.

2.2. Spectra

The absorption spectra were recorded on a Perkin-Elmer *Lambda 20* UV–Vis spectrophotometer. The polarization of the spectra was procured by using film polarizers. The assayed solutions of merocyanine in methanol contained the appropriate metal ions at the following final concentrations 10^{-4} M l^{-1}. Distilled water was purified with a Millipore Milli-Q Plus system. The concetration of water in the measured samples varied from 0 to 50% in the intervals of 12.5%. The final concetration of pure merocyanine and its mero-ion complexes in PVA film was 5×10^{-4} l^{-1}. The anisotropic properties of the samples were acquired by the mechanical stretching of the PVA film (up to 300% of the initial length) according to the following formula:

$$E = \frac{\Delta l}{l_0} \times 100\%$$

where Δl indicates absolute change in length and l_0 indicates length of unstretched film.

Two assays were performed for each analyzed sample. One, where the applied direction of the electric field vector was consistent with the direction that the PVA film was stretched (parallel component). The second, where the applied direction of the electric field vector was perpendicular to the direction that the PVA film was stretched (perpendicular component).

3. Results

Fig. 1 shows the structure of stilbazolium merocyanine in the free base form. In order to obtain this form, methanol solutions of various salts were added to a methanol solution of the dye. The pH of all solutions was monitored and maintained between 6.35 and 7.50. Only then was the appropriate amount of water added to the solutions. The absorption spectra of pure merocyanine and of the formed complexes are presented on Fig. 2. The band which appears at approximately 410 nm is related to the protonated form of the dye (curve 1, Fig. 2B). Two long-wave bands which appear at approximately 510 nm (Fig. 2A) and 550 nm (Fig. 2B) are related to the complex between merocyanine and ion [10].

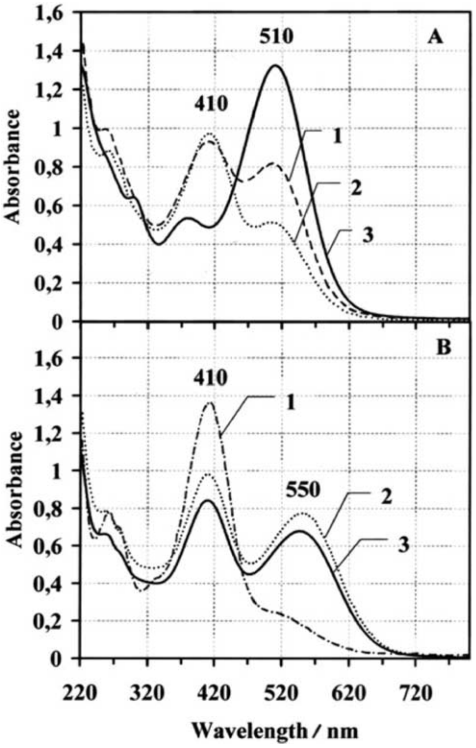

Fig. 2. The absorption spectra for methanol-solutions of mero-ion complexes in the presence of various anions: A. mero–Cu^{2+} complex in the presence of 1. SO_4^{2-} 2. Cl^-, 3. CH_3COO^-. B. mero–Co^{2+} complex in the presence of 1 merocyanine only, 2. Cl^-, 3. CH_3COO^-.

The comparative analysis of Fig. 2A and B indicates a stronger effect of Cu^{2+} ion on the formation of the mero–ion complex as compared to the effect of Co^{2+} ion on the formation of the respective mero–ion complex. The highest intensity of absorption was observed for the long-wave band corresponding to the mero–Cu^{2+} complex formed in the presence of CH_3COO^- (curve 3, Fig. 2A).

Fig. 3 illustrates the changes in the location of λ_{max} (Fig. 3A) and the intensity of absorption (Fig. 3B) of the mero–Cu^{2+} complex in accordance

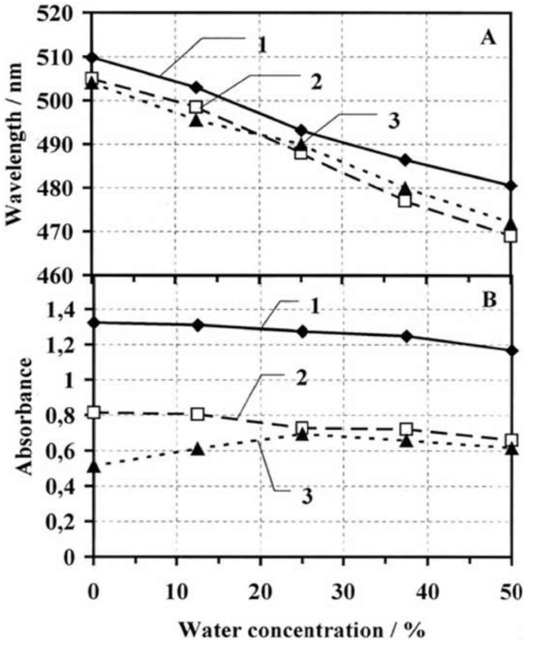

Fig. 3. The changes in the location of $\Delta\lambda_{max}$ (A) and intensity of absorption (B) for the mero–Cu^{2+} related band in accordance to the samples water concentration (%) and the presence of the following anions: CH_3COO^- (curve 1), SO_4^{2-} (curve 2), and Cl^- (curve 3).

to the water concentration (%) of the tested sample in the presence of one of three ions: CH_3COO^- (curve 1), SO_4^{2-} (curve 2), and Cl^- (curve 3). All three types of solutions showed a hypsochromic shift. However, the minor hipochromic effect was observed solely in the presence of CH_3COO^- and SO_4^{2-} anions.

In the presence of Cl^-, the initial minor increase in the intensity of absorption was registered at up to app. 25% of the water concentration. With higher water concentrations, a decrease in intensity of absorption was observed (Fig. 3B, curve 3).

Fig. 4 shows the location of λ_{max} (Fig. 4A) and the intensity of absorption (Fig. 4B) of the mero–Co^{2+} complex in accordance to the water concentration (%) of the analyzed samples, where curve 1 represents the complex in the presence of CH_3COO^- anions, and curve 2 represents the complex in the presence of Cl^- anions. The analysis of both curves reveals that in the presence of each anion the hypsochromic shift of λ_{max} and the hipochromic effect are taking place. The most visible changes were observed in samples containing up to 25% of water. Further increase in water concentration only slightly modified the absorption of the complex.

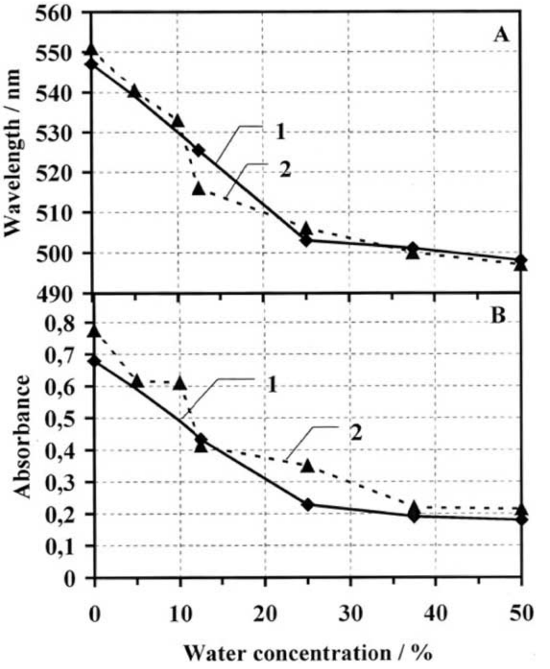

Fig. 4. Changes in the location of λ_{max} (A) and intensity of absorption (B) for the mero–Co^{2+} related band in accordance to the samples water concentration (%) and in the presence of the following anions: CH_3COO^- (curve 1) and Cl^- (curve 2).

The spectra of polarized absorption of pure merocyanine and complexes mero–Cu^{2+} and mero–Co^{2+} (all embedded in both isotropic and anisotropic PVA matrix) are presented in Fig. 5. The comparative analysis of the curves representing the isotropic matrix (Fig. 5A) with curve 1 of Fig. 2B and curve 3 of Fig. 2A and B has revealed that the shape of the spectra is not altered in the presence of PVA matrix. The spectra of polarized absorption for samples embedded in anisotropic PVA matrix are shown in Fig. 5B, C, and D. Symbol "‖" (parallel) indicates that the direction of mechanical deformation of the matrix (same as the direction of the PVA film stretching) corresponds to the parallel component of the electric vector ***E***. Symbol "⊥" (perpendicular) indicates that the direction of the PVA film stretching is perpendicular in relation to the direction of the electric vector ***E***. As shown on Fig. 5B, C, and D, intensity of absorption for the parallel component is higher than that of the perpendicular component. The linear dichroism (Table 1) of the polarized absorption spectra was calculated based on the following formula:

$$LD = \frac{A_{II} - A_{\perp}}{A_{II} + 2A_{\perp}}$$

where $A_{\parallel}$ is maximum of the intensity of absorption for the parallel component, and $A_{\perp}$ is maximum

Table 1
Linear dichroism (LD) of merocyanine, mero–Cu^{2+} complex, and mero–Co^{2+} complex, all embedded in PVA anisotropic film

	LD for λ_{max1}	LD for λ_{max2}
Merocyanine	0.33	0.18
Merocyanine–Cu^{2+}	0.14	0.22
Merocyanine–Co^{2+}	0.11	0.09

λ_{max1}—maximum of absorption for protonated form of the dye (at approximately 400 nm). λ_{max2}—maximum of absorption for basic form of the dye (at approximately 500 nm), mero–Cu^{2+} complex (at approximately 510 nm), and mero–Co^{2+} complex (at approximately 550 nm).

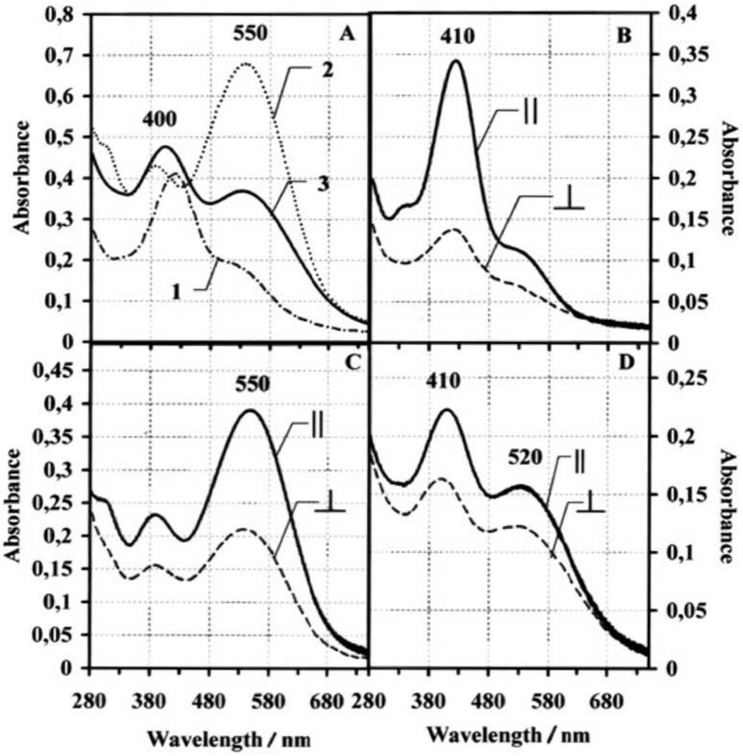

Fig. 5. The spectra of absorption for: A. embedded in isotropic PVA matrix; pure merocyanine (curve 1), complex mero–Cu^{2+} (curve 2) and mero–Co^{2+} (curve 3). B. pure merocyanine in anisotropic PVA matrix (300%), C. mero–Cu^{2+} in anisotropic PVA matrix (300%), D. mero–Co^{2+} in anisotropic PVA matrix (300%). Symbol "‖" indicates the parallel component, symbol "⊥" indicates the perpendicular component.

of the intensity of absorption for the perpendicular component.

Table 2 shows values $\Delta\lambda_{max} = \lambda_{max(0\%H_2O)} - \lambda_{max(50\%H_2O)}$ and $\Delta A_{max} = A_{max(0\%H_2O)} - A_{max(50\%H_2O)}$ representing respective anions participating in the analyzed Cu^{2+} and Co^{2+} complexes.

4. Discussion

The observed shift in the absorption bands of merocyanine dissolved in solution as compared to merocyanine embedded in PVA matrix results from the solvatochromic effect. However, there is a chance of dissociation of the Co^{2+} containing complexes during the embedding process into the PVA matrix. As a result of this dissociation, the higher level of the free basic form of merocyanine is reflected in the absorption spectrum. The comparison of absorption bands of pure merocyanine dissolved in solution with pure merocyanine embedded in PVA also illustrates this phenomenon. The change in location of absorption bands as dependent on the polarity of microenvironment are well known and documented [13].

The combined analysis of the value of ΔA_{max} (Table 2) and graphs presented on Fig. 3 and Fig. 4 shows that the hipochromic effect of the respective anions in the presence of H_2O is lower for the mero–Cu^{2+} complex as compared to the mero–Co^{2+} complex, indicating higher stability of the complex between dye and Cu^{2+} ion. These findings also demonstrate that the formation of the mero–Cu^{2+} complex depends more on the presence of various anions than the formation of the mero–Co^{2+} complex. The highest concentration of the mero–Cu^{2+} in the methanol solution was

Table 2
The changes in the location of $\Delta\lambda_{max}$ and ΔA_{max} for analyzed complexes in the presence of various anions

Anion	Merocyanine–Cu^{2+}		Merocyanine–Co^{2+}	
	$\Delta\lambda_{max}$ [nm]	ΔA_{max} [a.u.]	$\Delta\lambda_{max}$ [nm]	ΔA_{max} [a.u.]
CH_3COO^-	29	0.16	49	0.50
SO_4^{2-}	36	0.15	–	–
Cl^-	32	−0.10	54	0.56

observed in the presence of the following order of the respective anions $CH_3COO^- > SO_4^{2-} > Cl^-$.

The most complicated features were exhibited by the mero–Cu^{2+} complex in the presence of Cl^- anions. The observed increase in intensity of absorption for this complex in the presence of 25% H_2O indicates that the $CuCl_4^{2-}$ in an anhydrous medium shows higher stability as compared to the complex including merocyanine. The increasing concentration of water (25%) intensifies the concentration of the mero–Cu^{2+} complex and consequently strongly indicates attenuation of the Cu–Cl bond. The attenuation of this bond clearly favors the formation of mero–Cu^{2+} complex and results from the increase in polarity of the solution.

The increase in water concentration above 25% generates an increase in the polarity of the media followed by an increase in the concentration of free H^+ ions. The final effect of this sequence of events is the shift of the acid–base equilibrium favoring the competitive reaction of protonating the basic form of merocyanine and thus confirming the previous observations [9,10]. In the presence of CH_3COO^- ions the solution, the described effect is not observed probably due to the low dissociation degree of acetic acid, which is responsible for the low concentration of free H^+ ions.

The values for linear dichroism of the λ_{max1} band (approximately 400 nm) presented in Table 1 indicate that in the presence of the complexes the lower degree of order of the protonated form of merocyanine embedded in the PVA matrix was observed. The LD values for λ_{max2} band (approximately 550 nm) shown in Table 1 indicate that the degree of order of the mero–ion complex depends on the type of the metal present in the complex suggesting the differentiation in the geometry of the complexes. LD for λ_{max1} and λ_{max2} refers to the complexes embedded in the PVA matrix of pH 6.8. The two processes, one, the protonization of merocyanine and, two, the forming of the complex are competitive to each other. The polar basic medium constitutes the most preferable conditions for the complex forming process therefore indicating that the complex with ion generates zwitterions. The molecule-orientation of the dye was disturbed the most in the presence of Co^{2+} ions (Table 1, Fig. 5D). The dissociation level due to the presence of water was app. 4-times higher for the mero–Co^{2+} complex than for the mero–Cu^{2+} complex (Table 2).

5. Conclusions

1. The solid geometry of the merocyanine-ion complexes depend on the type of the metal participating in the complex. The very nature of the geometry of these complexes directly affects their stability in the presence of water and various anions.
2. The use of merocyanine as an indicator of the presence of Cu^{2+} and Co^{2+} ions remains restricted only in a very closely controlled environment.
3. The complex-formation efficiency of the mero–Cu^{2+} complex strongly depends on the presence of various anions ($CH_3COO^- > SO_4^{2-} > Cl^-$). The same dependence of the mero–Co^{2+} complex is less severe.
4. The degree of order of the protonated form of the dye is reduced by the presence of mero–ion complexes.

Acknowledgements

This study was financially supported by Poznañ University of Technology grant PB-62-199/2004-BW.

References

[1] Hippeli S, Elstner EF. Transition metal ion-catalyzed oxygen activation during pathogenic processes. FEBS Letters 1999;443:1–7.

[2] Buranaprapuk A, Leach StP, Kumar ChV, Bocarsly JR. Protein cleavage by transition metal complexes bearing amino acid substituents. Biochim Biophys Acta 1998;1387:309–16.

[3] Vanoppen DL, Jacobs PA. Cobalt-containing molecular sieves as catalysts for the low conversion autoxidation of pure cyclohexane. Part II. A kinetic study. Catalysis Today 1999;49:177–83.

[4] Baughman GL. Fate of copper in copper-complexed dyes during biological waste treatment III. Dyes and Pigments 2001;48:179–86.

[5] Niedbalska M, Gruda I. Effect of substituents on the solvatochromism of stilbazolium merocyanines. Can J Chem 1990;68(5):691–5.

[6] Gruda I, Pagé M, Bolduc F, Laliberté St, Noel Ch. Merocyanines: potential new anticancer agents. Anticancer Research 1987;7:1125–8.

[7] Wiktorowicz K, Niedbalska M, Planner A, Frąckowiak D. Incorporation of stilbazolium merocyanines into human leukocytes measured by flow cytometry, A. Biochim Pol 1995;42(3):333–8.

[8] Cofta J, Niedbalska M, Kostrzewa A, Cegielski R, Manikowski H, Wiktorowicz IE. Application of stilbazolium merocyanines for fluotescent staining of human peripheralblood leukocytes. In: Slavik J, editor. Fluorescence Microscopy and Fluorescent Probes, Vol. II. New York: Plenum Press; 1998. p. 223–7.

[9] Manikowski H, Niedbalska M, Cegielski R. EPR of copper–merocyanine complexes. Molecular Physics Reports 2000; 28:85–8.

[10] Cegielski R, Niedbalska M, Manikowski H. Interactions of stilbazolium merocyanine with transition metal ions. Dyes and Pigments 2001;50:35–9.

[11] Gruda I, Bolduc F. Spectral properties and basicity of stilbazolium betaines containing bulky substituents on the quinoid ring. J Org Chem 1984;49(18):3300–5.

[12] Fiksiński K, Frąckowiak D. Comparison of various films used in biophysical investigations as anisotropic matrices. Spectroscopy Lett 1980;13(12):873–89.

[13] Brooker LGS. Some recent developments in the chemistry of photographic sensitizing dyes. Exper Suppl 1955;II(17): 229–57.