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Analysis of spectral properties of stilbazolium merocyanine complexes with Fe(II) and Fe(III) ions

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Abstract

The potential use of merocyanine dye (1-(12-hydroxydodecyl)-4-[(3-hydroxy-4-oxocyclohexa-2,5-dienylidene)-ethylidene]-1,4-dihydropyridine) was investigated as an effective monitoring tool for the presence of free iron ions. We show that merocyanine forms stable complexes with both Fe^{2+} and Fe^{3+} ions provided that the pH level is maintained at 10. Only the basic form of merocyanine was capable of forming these complexes. Complexes between merocyanine and iron ions demonstrated some features of charge-transfer complexes and did not dissociate in the presence of water.

Keywords: Stilbazolium merocyanine; Fe^{2+} and Fe^{3+} ions; Absorption; Fluorescence; Complexes

1. Introduction

As a result of the development of industrial civilization, a progressive polluting and contamination of the environment is observed. Numerous contaminants containing toxic transition metals slowly but constantly penetrate the soil, atmosphere and water. These contaminating metals do not decompose, but rather are progressively absorbed by the cells of living organisms [1]. Though iron is an indispensable metal in the respiration process, its slow accumulation followed by an increase of its concentration in the system can cause many serious diseases. Diabetes, certain kinds of cancers and rheumatic joint inflammation are among the many disorders potentially attributed to the hydroxyl radical [2].

The formation of hydroxyl radical in so-called Fenton reaction is directly catalyzed by iron ion [3]. Therefore, monitoring of the iron ion concentration in the environment and the cells of organisms is of great importance. Numbers of organic compounds capable of forming complexes with iron ions are utilized as a useful monitoring tool [4]. Complexes formed between these compounds and iron ions cause measurable changes in the absorption spectra of UV/vis light and fluorescence, allowing both qualitative and quantitative evaluations [5].

As was presented in the previous publications [6,7], stilbazolium merocyanine containing the hydroxyl group in the *ortho* position in relation to the carbonyl group in the quinonoid ring is an effective indicator of the presence of transition

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metals ions. It can also be incorporated into different types of human peripheral blood leukocytes. Depending on the type of ion (Cu^{2+}, Co^{2+} or Fe^{3+}) in complex with dye, the appearance of the specific absorption bands at various wavelengths was observed [6]. The high affinity of analyzed dye to the cellular membranes also ranks as one of its important features.

The spectral characterization of merocyanine complexes with Fe^{2+} and Fe^{3+} ions constitutes the subject of this paper.

2. Materials and methods

2.1. Chemicals

1-(12-Hydroxydodecyl)-4-[(3-hydroxy-4-oxocyclohexa-2,5-dienylidene)-ethylidene]-1,4-dihydropyridine (Me) was used as the dye (Fig. 1) and its synthesis is described elsewhere [8]. Melting point, IR and ^1H NMR spectra of merocyanine were measured previously [7].

$FeCl_2{*}4H_2O$ (pure p.a.) from POCh-Gliwice (Poland) and $FeCl_3$ anhydrous (purum; >98%) from Fluka Chemika were used as sources of Fe^{2+} and Fe^{3+} ions without further purification.

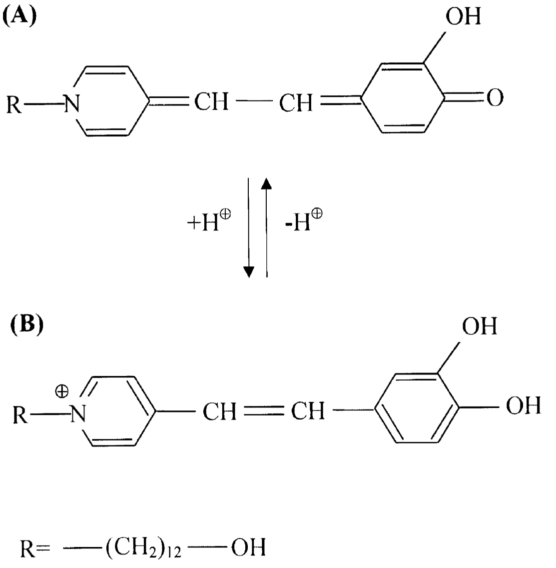

Fig. 1. Structures of merocyanine in its free basic form (A) and protonated form (B).

All samples were dissolved in methanol (p.a.) from POCh-Gliwice. Destilled water was purified with a Millipore Milli-Q Plus system.

The pH of each measured sample was controlled. An addition of 0.2 M NaOH (POCh-Gliwice) to solutions of Fe ions allowed formation of merocyanine-ion complex in 1:1 ratio.

2.2. Spectra

Absorption spectra were recorded on a Perkin-Elmer *Lambda 20* UV/vis spectrophotometer. The final concentrations of Me–Fe complexes in measured solutions were 10^{-4} and 10^{-3} M l^{-1}. Whereas final concentrations of a water in the samples were changed as follow: 0, 12.5, 25, 37.5, 50% (v/v).

The fluorescence spectra were measured with a fluorescence spectrophotometer Hitachi *F-4500*. Final concentrations of both the Me–Fe complex and pure merocyanine were 10^{-4} M l^{-1}. Wavelengths of excitation were 400 and 500 nm.

3. Results

The stilbazolium merocyanine can occur in two different forms depending on the polarity of the microenvironment [8,9]. One of them is a protonated form and the second one is a free base (Fig. 1).

Fig. 2 shows changes in the location of the absorption band (Fig. 2A) and the absorption intensity in relation to water concentration. Curves marked "1" are related to concentration 10^{-4} M l^{-1} of the merocyanine-Fe^{2+} complex, curves marked "2" are related to concentration 10^{-3} M l^{-1} of the same complex. The hipsochromic shift was observed for both analyzed concentrations. For 10^{-3} M l^{-1} concentration of the complex in the presence of 50% of water, a batochromic shift was observed. A new long wave band appearance at approximately 630 nm was generated by an increase in the water concentration. The observed occurrence of the long wave band could reflect a formation of micelle-type association of monomers as has been previously observed [9]. The precipitation of the complexes

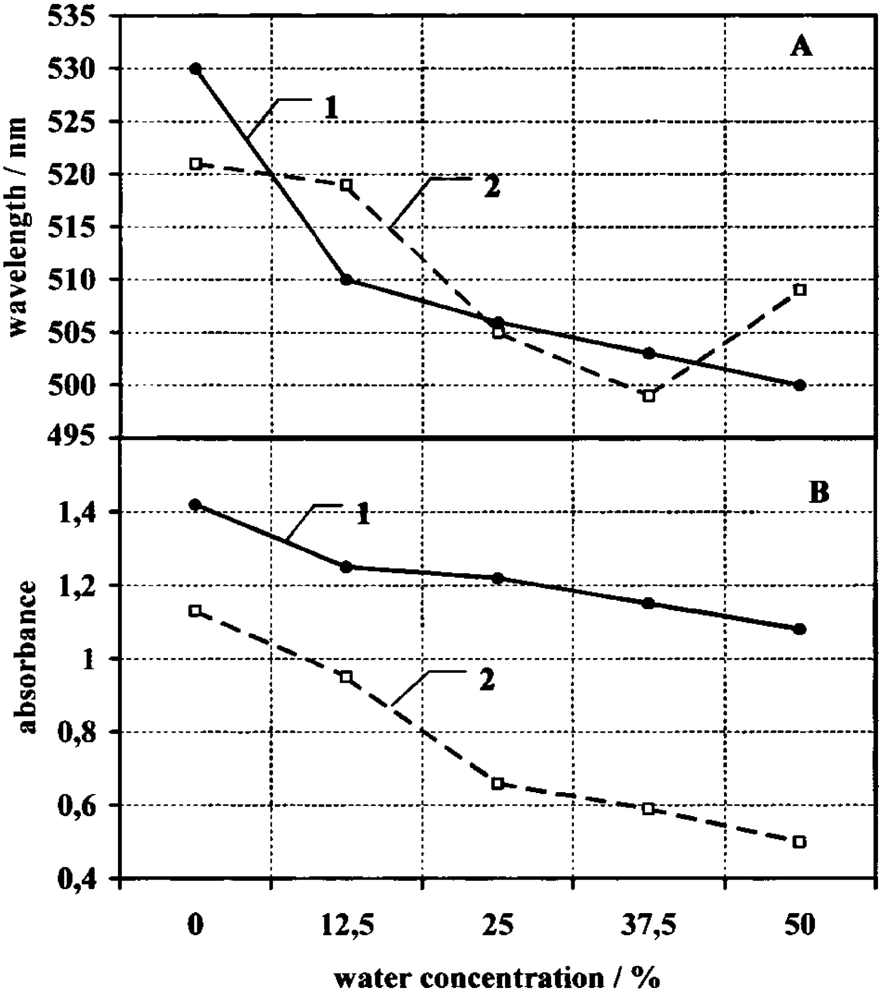

Fig. 2. Changes in location of the absorption band (A) and maximum of absorption intensity (B) for merocyanine–Fe^{2+} complex as a function of water concentration. Curve 1. Complex concentration 10^{-4} M l^{-1}; Curve 2. Complex concentration 10^{-3} M l^{-1}.

was observed at higher water concentrations. It caused both a decrease of absorption intensity of the major complex band (Fig. 2B) and a change in the shape of the complex spectrum as compared to the spectra of complexes not containing water.

Fig. 3 illustrates the same relation as presented in Fig. 2, except for complexes merocyanine–Fe^{3+} where Fig. 3A represents the location of the band and Fig. 3B represents the intensity of absorption in relation to the water concentration. Occurrence of the appearance of a new long wave absorption band and the decrease of absorption intensity as a result of precipitation similar to merocyanine–Fe^{2+} complex was also observed for merocyanine–Fe^{3+} complex.

After precipitation followed by several washes with water and then desiccation, the complexes were re-suspended in methanol. The absorption spectra of solutions of the complexes prepared as described above are shown in Fig. 4 where the shown curves represent respectively: curve 1 the spectrum of pure merocyanine, curve 2 the spectrum of the merocyanine–Fe^{3+} complex, and curve 3 the spectrum of the merocyanine–Fe^{2+} complex. As shown in Fig. 4, the spectra of the complexes between merocyanine and the respective ion are

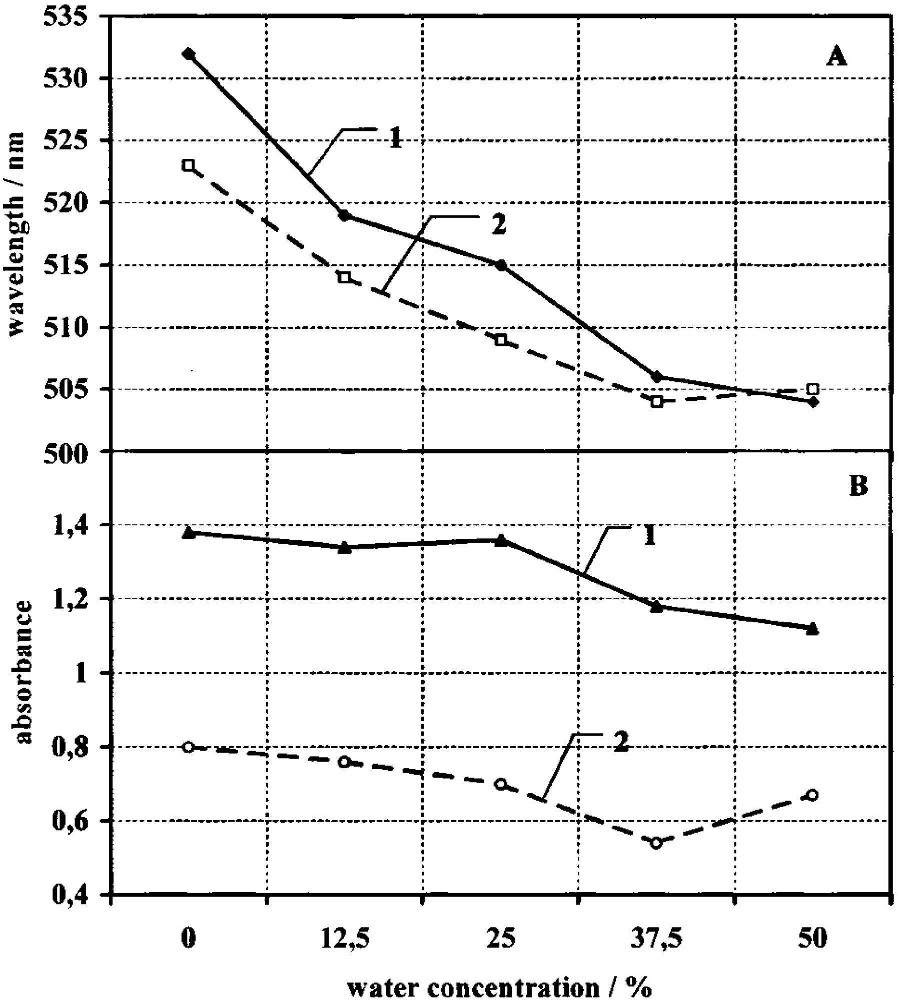

Fig. 3. Changes in the location of the maximum of absorption (A) and the maximum of absorption intensity (B) for merocyanine–Fe^{3+} complex as a function of water concentration. Curve 1. Complex concentration 10^{-4} M l^{-1}; Curve 2. Complex concentration 10^{-3} M l^{-1}.

slightly different in location and shape (curves 2 and 3).

Fig. 5A shows a fluorescence spectra of the protonated form of merocyanine with absorption at 400 nm, and the basic form of merocyanine with absorption at 500 nm. Merocyanine in the protonated form shows a maximum of fluorescence at 572 nm, while merocyanine in the basic form emitted a fluorescent light at 602 nm.

Fig. 5 shows fluorescence of merocyanine in the presence of Me–Fe^{2+} (5B) and Me–Fe^{3+} complex (5C). A slight reduction of the intensity of luminosity (Fig. 5B and C) for both types of complexes was observed, as compared to the intensity of the fluorescence of pure merocyanine (Fig. 5A).

4. Discussion

The merocyanine dye analysed in this paper was synthesized with a future medical use in mind. As we have shown here, merocyanine in complex with Fe ions shows much different features as compared to Me–Cu^{2+} and Me–Co^{2+} complexes [6].

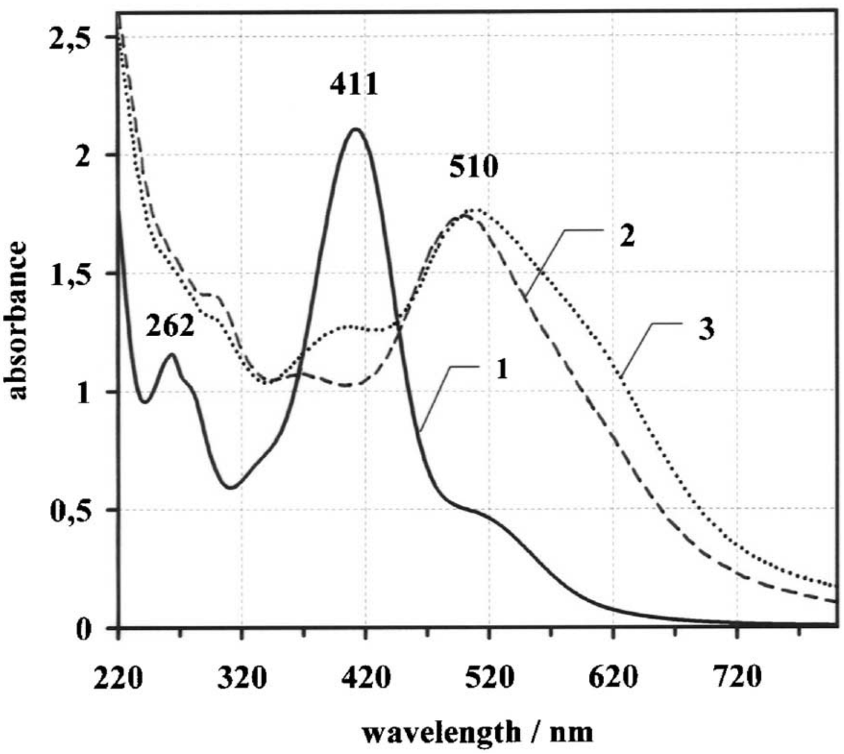

Fig. 4. Absorption spectra of the methanol solutions (complexes were first precipitated and then resuspended in methanol at a concentration approximately 10^{-4} M l^{-1}). Curve 1. Pure merocyanine; Curve 2. Merocyanine–Fe^{2+} complex; Curve 3. Merocyanine–Fe^{3+} complex.

The instability of Me–Fe complexes is related to a drastic pH decrease of the solutions following an increase in the concentration of Fe ions. This observation led us to a further analysis of those complexes in very closely controlled pH.

The Me–Fe complex shows a negative solvatochromism as a result of the increase in water concentration, similarly to various derivatives of the stilbazolium betaine type (Figs. 2 and 3) [9]. Results presented in this paper show that maintaining pH at the constant value of approximately 10 assures stability of the complex by effectively inhibiting its competitive reaction of dye protonation. The presented data also shows that only the basic form of merocyanine dye is capable of forming complexes with Fe ions due to a decrease in the fluorescence intensity of the basic form of merocyanine by approximately an order of 1 of magnitude in the presence of Fe ions. Whereas, the fluorescence intensity of the protonated form decreases by approximately two times in the presence of Fe^{2+} ions and approximately four times in the presence of Fe^{3+} ions. In the presence of polar solvent of pH = 10, the protonated and basic forms of merocyanine reach an equilibrium causing a decrease in fluorescence intensity of the protonated form. The Me–Fe complexes do not emit fluorescent light since the presence of Fe ions does not affect either the shapes or positions of maxima of their fluorescent spectra (Fig. 5).

At a low pH, complexes between merocyanine and Fe ions are not formed as described earlier [6]. The increase of the polarity of the medium by the addition of water did not affect the stability of the complex, but merely facilitated its aggregation followed by precipitation (Fig. 4). The presence of Fe ions is responsible for the termination or reduction to various degrees of merocyanine fluorescence

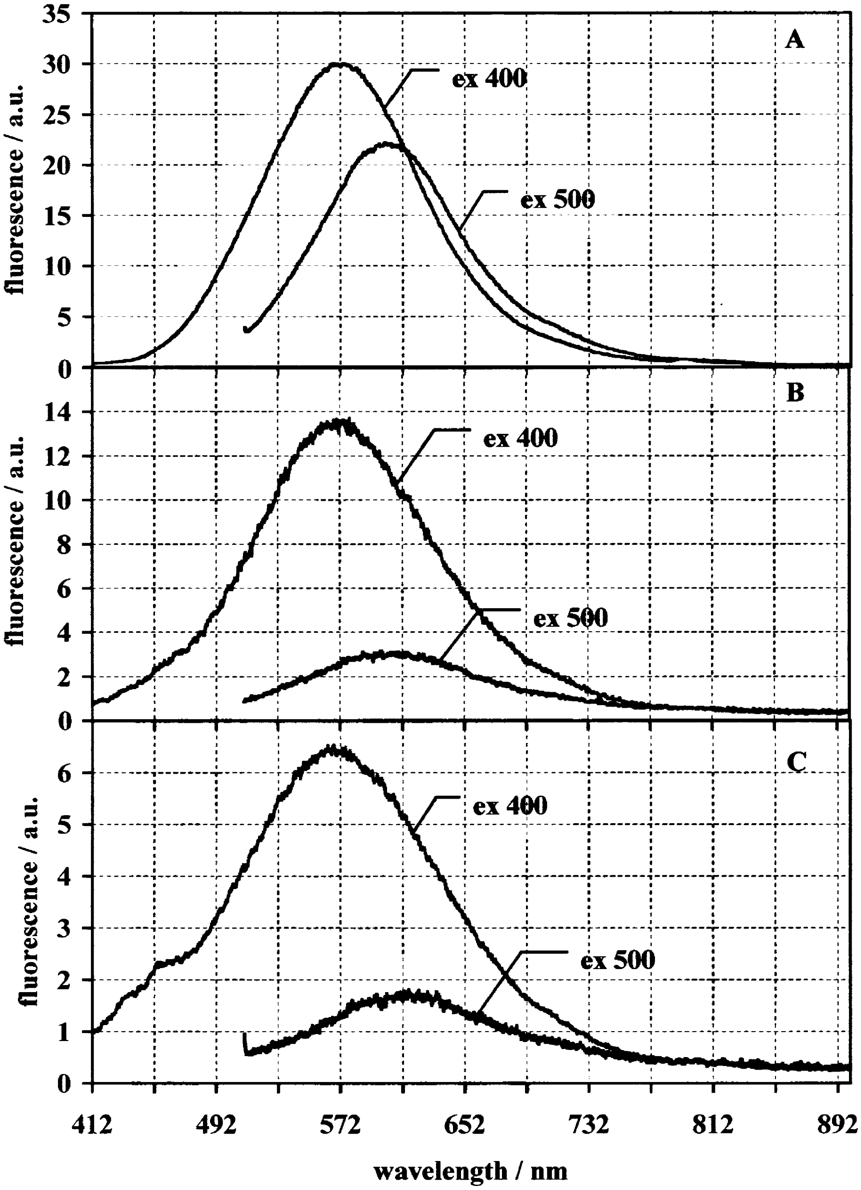

Fig. 5. Fluorescence spectra of the methanol solutions, final samples concentrations were 10^{-4} M l^{-1} at excitation 400 and 500 nm. A. Pure merocyanine; B. Merocyanine–Fe^{2+} complex; C. Merocyanine–Fe^{3+} complex.

depending on the oxidation level of Fe ions, as shown in Fig. 5. The decrease in the observed intensity of luminosity of the protonated form results from a lower concentration of this form in solutions of Me–Fe complex. The decrease in the observed intensity of the light emission by the basic form shows that the formed complexes can have a charge-transfer quality causing termination of fluorescence. Fe^{3+} ions reduce the fluorescence of merocyanine twofold stronger in comparison with Fe^{2+} ions. This feature can result from a larger shift of electrons π of double linked bonds in the merocyanine molecule towards the Fe ions with a higher oxidation degree.

5. Conclusions

1. The data presented in this paper shows that complexes between merocyanine and Fe ions are stable and do not dissociate in the presence of water.
2. Complexes containing Fe^{3+} as well as Fe^{2+} demonstrate features of charge-transfer complexes and are formed only with the basic form of merocyanine. These complexes do not emit fluorescent light.
3. Both forms of merocyanine emit fluorescent light with the maxima of 572 nm (protonated form) and 602 nm (basic form).
4. The derivative of stilbazolium merocyanine analysed in this paper can serve not only as a indicator of the presence of free Fe ions in the basic medium, but can also be used as an agent in removing excesses of these ions from solutions.

Acknowledgements

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References

[1] Reimann C, Koller F, Kashulina G, Niskavaara H, Englmaier P. Influence of extreme pollution on the inorganic chemical composition of some plants. Environmental Pollution 2001;115:239–52.

[2] Domı́ngeuez-Rosales JA, Mavi G, Levenson SM, Rojkind M. H_2O_2 is an important mediator of physiological and pathological healing responses. Archives of Medical Research 2000;31(1):15–20.

[3] Xie Y, Chen F, He J, Zhao J, Wang H. Photoassisted degradation of dyes in the presence of Fe^{3+} and H_2O_2 under visible irradiation. J Photochem Photobiol A: Chem 2000;136:235–40.

[4] Shigeyuki Y, Yoshihiko F, Yutaka H, Hiroyuki N. Synthesis, structure, and complexation properties with transition metal cations of a novel methine-bridged bisquarylium dye. Dyes and Pigments 2002;52(4):245–52.

[5] Tarazi L, Narayanan N, Sowell J, Patonay G, Strekowski L. Investigation of the spectral properties of a squarylium near-infrared dye and its complexation with Fe(III) and Co(II) ions. Spectrochimica Acta Part A 2002;58:257–64.

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

[7] Cofta J, Niedbalska M, Kostrzewa A, Cegielski R, Manikowski H, Wiktorowicz KE. Application of stilbazolium merocyanines for fluorescent staining of human peripheral blood leukocytes. In: Slavik J, editor. Fluorescence microscopy and fluorescent probes, vol. II. New York: Plenum Press; 1998. p. 223–7.

[8] 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.

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