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Synthesis and colour spectrophotometric measurements of some novel merocyanine dyes

H.A. Shindy*, M.A. El-Maghraby, Fayez M. Eissa

Department of Chemistry, Faculty of Science at Aswan, South Valley University, Aswan, Egypt

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Novel merocyanine dyes cyanine dyes derived from benzo[2,3-b; 2′, 3′ - b′]bis pyrazolo[4,5-b]-l,4-(oxa-, thia-, and pyra)-zine-6,12-dione ring system were prepared. The prepared cyanines involve acyclic merocyanine and cyclic merocyanine types. Colour spectrophotometric measurements for all the synthesized merocyanine dyes were investigated in 95% ethanol solution. Colour spectrophotometric measurements for some selected synthesized merocyanines were examined in pure solvents having different polarities and/or in aqueous universal buffer solutions. General chemical–physical characterization was carried out through elemental analysis, IR and ^{1}H NMR spectral data.

1. Introduction

Cyanine dyes have been used for many years in various applications [1–16], such as silver halide sensitizers in photographic industry, laser technology, immunoassays and flow cytometry, and for labeling biomolecules for a variety of applications such as DNA sequencing. Scientists favor using cyanine dyes in biological applications because, among other reasons, many of the dyes operate in the near IR (NIR) region of the spectrum (600–1000 nm). This makes these cyanine dyes less susceptible to interference from autofluorescence of biomolecules. Besides, merocyanine dyes have practical application ranging from physics to medicine and biology [17–20]; for example, they are extensively used as nonlinear optical (LNO) materials, photosensitizers in the photodynamic treatment of cancer, radiosensitizers in solid tumor treatment, diagnostic agents in medicine and potentiometric sensors. Owing to their pronounced solvatochromic effect, merocyanine dyes have found widespread utilization as probes for solvent polarity [21]. In addition, merocyanine dyes are characterized by a large area of high-tech applications, such as their application in procedures for disinfection of blood products [22]. Taking in accounts and consideration the above vital and important benefits of cyanine dyes, we prepared here some new solvatochromic and halochromic merocyanine dyes as new synthesis contribution and spectroscopic investigation in this field, and/or to be used in any of the various application fields of cyanine dyes, particularly as photographic sensitizers, as indicators for solvent polarity and as indicators for acid–base titration in analytical chemistry. Also, we hope that this paper will be interesting, informative and stimulating not only for cyanine dyes chemists, but also for scientists in other fields like biochemistry, physics, pharmacology, medicine and for all whom interested in the light absorbing systems in their research, labeling biomolecules and/or in the synthesis and characterization of complex organic compounds.

2. Results and discussion

2.1. Synthesis

Reaction of 4,10-diformyl-2,8-diphenyl-5,ll-dihydro-benzo[2,3-b; 2′, 3′ - b′]bis pyrazolo [4,5-b]-l,4-(oxa-, thia-, and pyra-)zine (1a–c) [23] with equimolar (bimolar) ratios of acetone, acetylacetone, or ethylacetoacetate in ethanol as organic solvent containing piperidine as a basic catalyst under refluxing conditions resulted the acyclic merocyanine (bis-acyclic merocyanine*)* dyes 2a–e (3a–e), Scheme 1, Table 1.

Chemical confirmation takes place for bis acyclic merocyanine dyes 3a–e, through the reactions of the acyclic merocyanine dyes

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* Corresponding author.
E-mail address: hashindy@hotmail.com (H.A. Shindy).

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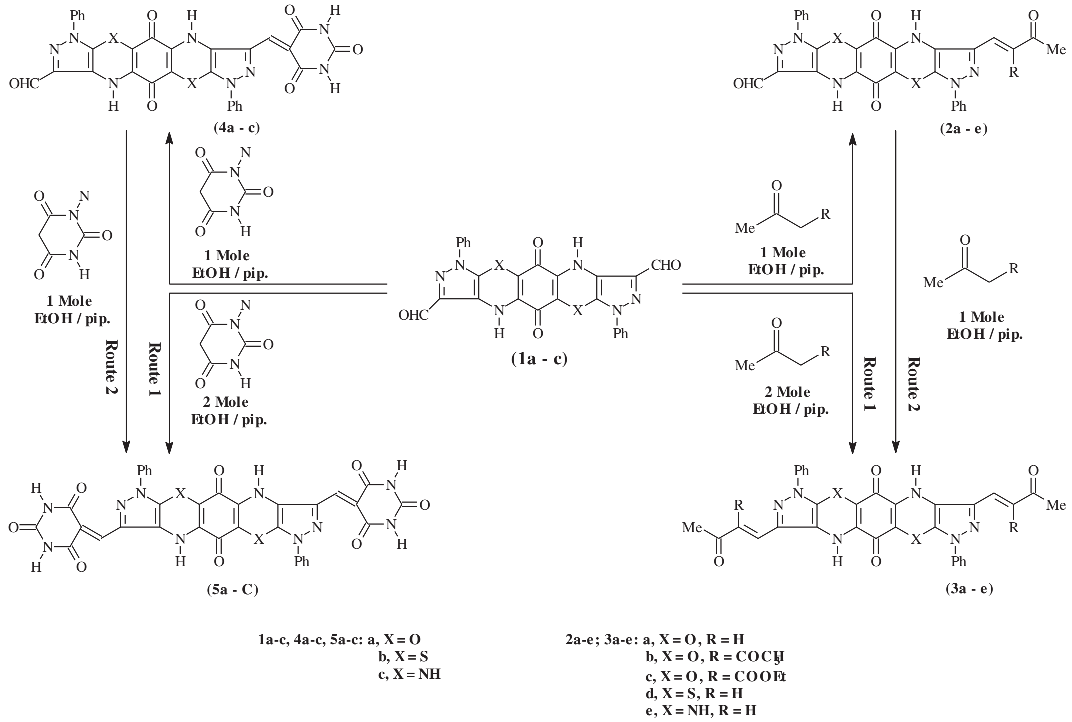


Scheme 1.

2a–e with equimolar ratios of the acetyl compounds (acetone, acetylacetone, or ethylacetoacetate) in ethanol containing piperidine to give the same bis acyclic merocyanine dyes 3a–e obtained via Route 1, characterized by the same melting points, mixed melting points, same IR and ^{1}H NMR spectral data, Route 2, Scheme 1.

Additionally, reaction of equimolar (bimolar) ratios of barbitone with the diformyl compounds 1a–c in ethanol as organic solvent

Table 1
Characterization of the prepared compounds.

Comp. No.	Colour of crystals	Molecular Formula (M. Wt.)	M P °C	Yield %	Analysis % (calcd/found)			Absorption Spectra in 95% Ethanol	
					C	H	N	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})
2a	Reddish brown	$C_{29}H_{18}I_2N_6O_6$ (546.5)	190–192	39	63.74 63.70	3.32 3.29	15.38 15.39	447	13920
2b	Bright violet	$C_{31}H_{20}$I2N6O6 (588.5)	195–197	71	63.27 63.32	3.43 3.44	14.28 14.30	461	12710
2c	Reddish brown	$C_{32}H_{22}I_2N_6O_8$ (618.6)	187–189	77	62.14 62.11	3.58 3.60	13.59 13.59	453	12130
2d	Violet	$C_{29}H_{18}N_6O_4S_2$ (578.6)	201–203	68	60.20 60.22	3.14 3.17	14.52 14.50	456	15610
2e	Pale violet	$C_{29}H_{20}N_8O_4$ (544.5)	209–211	63	63.97 64.00	3.70 3.68	20.58 20.55	468	15910
3a	Reddish brown	$C_{32}H_{22}N_6O_6$ (586.6)	203–205	59	65.53 65.55	3.78 3.76	14.33 14.39	454	14110
3b	Bright violet	$C_{36}H_{26}N_6O_6$ (670.6)	202–204	68	64.47 64.46	3.91 3.88	12.53 12.55	470	16100
3c	Reddish brown	$C_{38}H_{30}N_6O_{10}$ (730.7)	167–169	78	62.46 62.48	4.14 4.13	11.50 11.47	464	14910
3d	Violet	$C_{32}H_{22}N_6O_4S_2$ (618.7)	213–215	74	62.12 62.09	3.58 3.60	13.58 13.60	472	15910
3e	Pale violet	$C_{32}H_{24}N_8O_4$ (584.6)	215–217	78	65.75 65.77	4.14 4.17	19.17 19.19	487	17010
4a	Violet	$C_{30}H_{16}N_8O_8$ (616.5)	188–190	39	58.45 58.39	2.62 2.66	18.18 18.21	501	17120
4b	Bright violet	$C_{30}H_{16}N_8O_6S_2$ (648.6)	207–209	42	55.55 55.56	2.49 2.51	17.28 17.29	509	17820
4c	Intense violet	$C_{30}H_{18}N_{10}O_6$ (614.5)	200–202	51	58.63 58.60	2.95 2.93	22.79 22.83	517	18010
5a	Violet	$C_{34}H_{18}N_{10}O_{10}$ (726.6)	197–199	71	56.20 56.19	2.50 2.47	19.28 19.30	509.5	20010
5b	Pale violet	$C_{34}H_{18}N_{10}O_8S_2$ (758.7)	211–213	83	53.82 53.80	2.39 2.35	18.46 18.42	515	19610
5c	Intense violet	$C_{34}H_{20}N_{12}O_8$ (724.6)	209–211	82	56.36 56.34	2.78 2.77	23.20 23.16	527	21310

Table 2
Colour spectrophotometric measurements for the merocyanine dyes (3b); (5a) in pure solvents.

Solvent	H_2O		EtOH		DMF		$CHCl_3$		CCl_4		Dioxane	
Dye No.	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})	λ_{max} (nm)	ε_{max} (L mole^{-1} cm^{-1})
(3b)	467	12530	470	16100	437	13220	422	11440	425	12980	429	12530
	–	–	–	–	491	16840	475	10720	481	10590	484	10280
(5a)	505	16940	509.5	20010	527	21120	518	15050	520	12310	524	11060

containing piperidine as a basic catalyst under refluxing conditions achieved the cyclic merocyanine (bis cyclic merocyanine) dyes 4a–c (5a–c), Scheme 1, Table 1.

Chemical confirmation is obtained for the bis cyclic merocyanine dyes 5a–c through the reactions of the cyclic merocyanine dyes 4a–c with equimolar ratios of barbitone in ethanol containing piperidine to give the same bis cyclic merocyanine dyes 5a–c obtained via Route 1, characterized by the same melting points, mixed melting points, same IR and ^{1}H NMR spectral data, Route 2, Scheme 1.

The structure of the prepared compounds was determined by elemental analysis, (Table 1), IR [24] and ^{1}H NMR [25] spectroscopy, Table 4.

2.2. *Colour spectrophotometric measurements*

2.2.1. *a - In 95% ethanol solution*

Colour spectrophotometric measurements for all the prepared merocyanine dyes were made by measuring their electronic visible absorption spectra in 95% ethanol solution. The dyes are thought to be deeper in colour when they absorb light at longer wavelength (bathochromic shifted and/or red shifted dyes), and consequently the lightining of the dye colour increase when they absorb light at shorter wavelength (hypsochromic shifted and/or blue shifted dyes). So, we may say that the colour of one dye is deeper than the other one if the wavelength of the maximum of absorption spectrum of the former is longer than that of the latter one [26].

The acyclic merocyanine dyes (bis-acyclic merocyanine dyes) 2a–e (3a–e) showed and disclosed electronic absorption spectra in 95% ethanol in visible region 447–468 nm (454–487 nm) where their band positions and molar extinction coefficients are largely affected by both the nature of the side group (R), the benzbiheterocyclic ring system present in the dye molecule, and the number of electronic charge transfer pathways inside the dye molecule, Table 1, Scheme 1. Thus, substituting R = H in dyes 2a (3a) by R = $-COCH_3$, -COOEt to obtain dyes 2b,2c (3b,3c), respectively, causes bathochromic shifts for the spectral absorption bands, Table 1, Scheme 1. This can be related to the presence of additional carbonyl groups in the latter dyes, which leads to additional electronic charge transfer pathways inside the dye molecules. In addition, substituting the methyl group in dyes 2b (3b) by the ethoxy group to obtain dyes 2c (3c) resulted blue shifted absorption bands with decreasing the intensities of these bands Table 1, Scheme 1. This can be attributed to the electron accepting character of the ethoxy group in dyes 2c (3c) and the electron releasing character of the methyl group in dyes 2b (3b). This clearly declare the role of the electron donating groups replaced on the carbonyl carbon, which enhances the electronic charge transfer, and the role of electron accepting groups which minimize the electronic charge transfer pathways inside the dye molecule. Also, it was observed that the oxazine dyes 2a (3a) gives hypsochromic shifted bands accompanied with decreasing the intensity of the bands if compared with the thiazine and pyrazine dyes 2d,2e (3d,3e). This can be due to the more electron attracting character of the oxazine ring compared with thiazine and pyrazine rings, Scheme 1, Table 1. Besides, it is noticed that, the electronic visible absorption spectra of bis-acyclic merocyanine dyes (3a–e) disclose bathochromic shifted and intensified bands if compared with their analogous acyclic merocyanine dyes (2a–c), Scheme 1, Table 1. This is due to the increase of the number of electronic charge transfer pathways in the bis-acyclic merocyanine dyes 3a–e, if compared with their analogous acyclic merocyanine dyes 2a–e, Scheme 2 (A), Table 1.

Additionally, it was found that the electronic visible absorption spectra bands and their molar extinction coefficients of the cyclic merocyanine (bis cyclic merocyanine) dyes 4a–c (5a–c) in 95% ethanol showed bathochromic and/or hypsochromic shifted bands with increase and/or decrease of the intensities of these bands, depending on the nature of the six membered heterocyclic nuclei and the number of the electronic charge transfer pathways inside the dye molecule, Schemes 1, 2 (B), Table 1. This can be related to the same reasons cited before in the case of the acyclic (bis-acyclic) merocyanine dyes 2a–e (3a–e).

2.2.2. *b - In pure solvents having different polarities*

Colour spectrophotometric measurements for some selected synthesized merocyanine dyes were made by measuring their electronic visible absorption spectra in pure solvents having different polarities. The dyes tend to give deeper colours and/or longer wavelength bands (bathochromic shifted and/or red shifted bands) in the solvents which have higher polarity and/or dielectric constants. So, we can say that, this solvent is more polar than the other one when the wavelength of the maximum of the absorption spectra bands of the dye in the former solvent is longer than in the latter one. Inversely, the dyes tend to give lighter colours and/or shorter wavelength bands (hypsochromic shifted and/or blue shifted bands) in the solvents which have lower polarity and/or dielectric constants. So we can say that this solvent is less polar than the other one when the wavelength of the maximum of the absorption spectra bands of the dye in the former solvent is shorter than in the latter one. Therefore, these cyanine dyes can be used as indicators for solvent polarity [27]. Deviation from this rule occurs when there are specific solvent interaction between the dye and solvent, like hydrogen bonding and/or molecular complex formation.

Table 3
The variation of absorbance with pH at fixed λ for the merocyanine dyes (3b); (5a) in different buffer solution.

		pH								
Dye		1.87	2.51	3.92	5.06	6.89	8.13	9.56	11.41	pKa
Absorbance at settled wavelength	(3b) λ 470 (nm)	0.900	0.932	1.180	1.280	1.360	1.550	1.700	1.730	4.240; 8.235
	(5a) λ 505 (nm)	1.275	1.306	1.423	1.617	1.755	1.947	2.009	2.074	8.655

Table 4
IR and ^{1}HNMR spectral data of the prepared dyes.

Dye	IR cm^{-1}	^{1}H NMR (DMSO) δ Assignment
2b	631,696 (-Ph); 1369 (C–N); 1678 (C=O quinone); 1758 (C=O); 3313 (NH cyclic).	4.28 (s, 6H, 2CH$_3$, 2COCH$_3$); 5.98 (s, 2H, 2NH); 6.84–7.98 (m, 11H, aromatic & CH =); 9.23 (s, 1H, CHO).
3b	679,770 (-Ph); 1371 (C–N); 1691 (C=O quinone); 1731 (C=O); 3281 (NH cyclic).	4.27 (s, 12H, 4CH$_3$, 4COCH$_3$); 5.86 (s, 2H, 2NH); 6.80–8.12 (m, 12H, aromatic & 2CH =).
4a	641,717 (-Ph); 1300,1375 (C–N); 1681 (C=O quinone); 1733 (C=O); 3401 (NH cyclic).	5.80 (s, 2H, 2NH); 6.54–8.57 (m, 13H, aromatic & 2CH =); 9.36 (s, 1H, CHO).
5a	633,682 (-Ph); 1323, 1361 (C–N); 1692 (C=O quinone); 1748 (C=O); 3424 (NH cyclic).	5.98 (s, 2H, 2NH); 6.52–8.42 (m, 16H, aromatic & 2CH = & 4NH).

The electronic visible absorption spectra of the bis acyclic merocyanine dye (3b) and bis cyclic merocyanine dye (5a) in pure solvents of different dielectric constant viz water (78.54), DMF (36.70), ethanol (24.3), chloroform (4.806), carbontetrachloride (2.238) and dioxane (2.209) [28] are recorded. The λ_{max} and ε_{max} values of the absorption bands due to different electronic transitions within the solute molecule in these solvents are represented in Table 2, Fig. 1 (A).

From Table 2 it is clear that, the electronic visible absorption spectra of the dyes (3b), (5a) in ethanolic medium are characterized by the presence of one or two essential absorption bands. These bands can be assigned to intramolecular charge transfer transitions [29]. These charge transfers are due to transfer of an electron lone pair from the N-Ph pyrazole nitrogen atom to the positively charged carbon atom of the carbonyl group, Scheme 2 (A), (B).

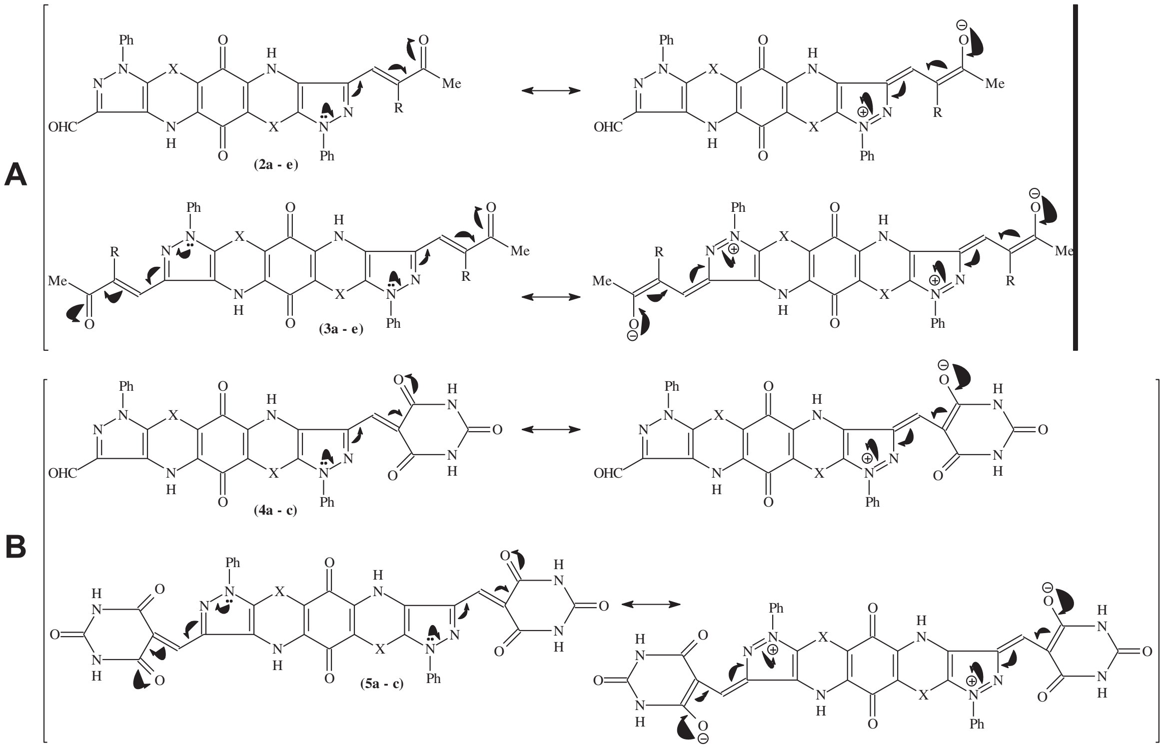
The data given in Table 2 show that the charge transfer band exhibits a hypsochromic shift in ethanol relative to DMF, dioxane, chloroform and carbontetrachloride. This effect may be attributed to the following factors :

a. The bathochromic shift in DMF relative to ethanol is a result of the increase in solvent polarity due to the increase of dielectric constant of DMF relative to ethanol.
b. The hypsochromic shift occurring in ethanol relative to dioxan, chloroform and carbontetrachloride is a result of the solute solvent interaction through intermolecular hydrogen bond formation between ethanol and the lone pair electrons of N-Ph pyrazole nitrogen atom, Scheme 3 (A). This slightly decreases the electron density on the N-Ph pyrazole nitrogen atom, and consequently decreases to some extent the mobility of the attached π electrons over the conjugated pathway to the positively charged carbon atom of the carbonyl group.

Also, from Table 2, it was observed the occurrence of unexpected hypsochromic shift with decreasing the intensity in the λ_{max} of the longer wavelength in water relative to ethanol. This can be mainly ascribed to the possible interaction of water molecules with the lone pair electrons of N-Ph pyrazole nitrogen atom, Scheme 3 (A). This makes difficult the charge transfer to the positively charged carbon atom of the carbonyl group, and consequently a hypsochromic shift is observed in water relative to ethanol.

2.2.3. c - In aqueous universal buffer solutions

Colour spectrophotometric measurements for some selected synthesized merocyanine dyes were examined by measuring their electronic visible absorption spectra in aqueous universal buffer



Scheme 2.

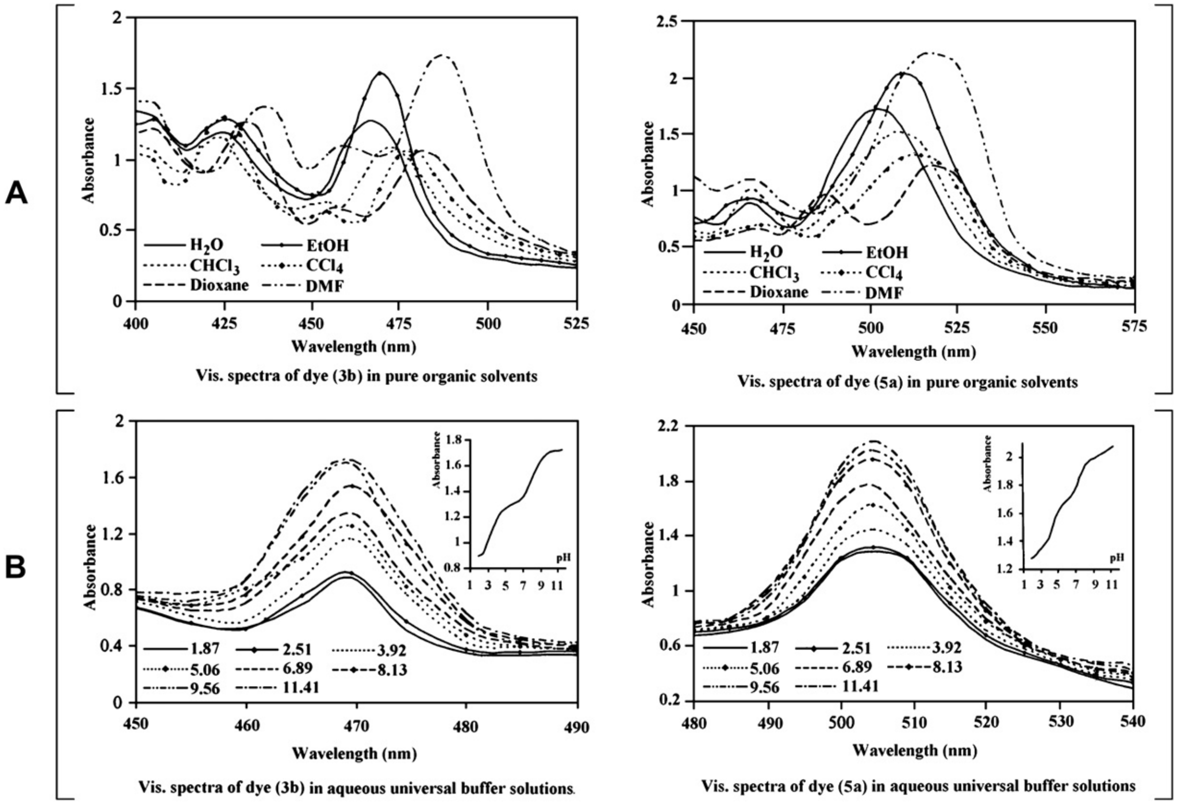
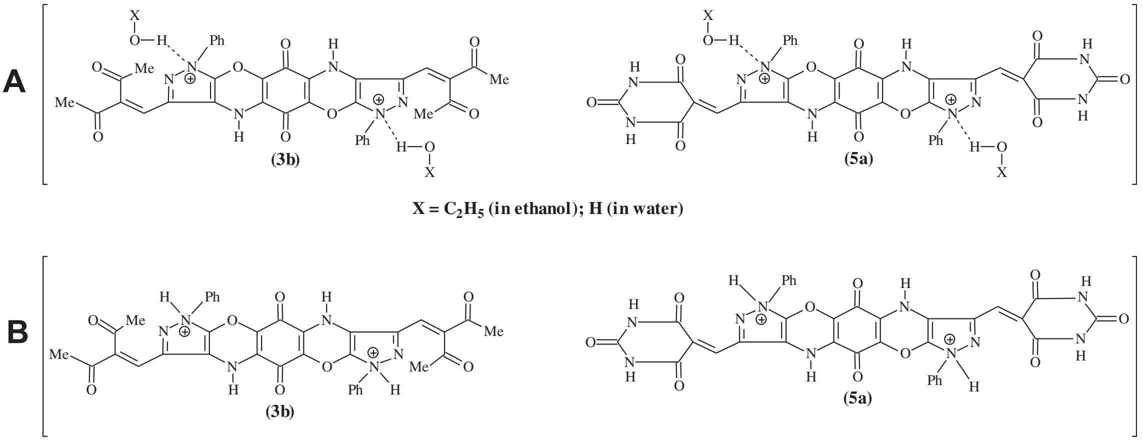


Fig. 1.

solutions. The dyes tend to give deeper colours and/or longer wavelength bands (bathochromic shifted and/or red shifted bands) in the buffer solutions having higher pH media and/or basic media. So, we can say that this buffer solution is more basic than the other one when the wavelength of the maximum of the absorption spectra bands of the dye in the former buffer is longer than in the latter one. Inversely, the dyes tend to give lighter colours and/or shorter wavelength bands (hypsochromic shifted and/or blue shifted bands) in the buffer solutions having lower pH media and/or acidic media. So, we can say that this buffer solution is more acidic than the other one when the wavelength of the maximum of the absorption spectra bands of the dye in the former buffer is shorter than in the latter one. Therefore, these cyanine dyes can be used as indicators for the pH of the buffer solutions and/or as indicators in acid–base titrations in analytical chemistry [30].

The solutions of the bis acyclic merocyanine dye (3b) and bis cyclic merocyanine dye (5a) have a permanent cationic charge in basic media which then discharged on acidification. This prompted and encouraged us to study their spectral behaviour in different buffer solutions in order to select a suitable pH for the use of these dyes as photosensitizers. The acid dissociation or protonation constant of these dyes have been determined. The effect of the compounds as photosensitizers increase when they are present in the ionic form, which has a higher planarity [31], and therefore more conjugation.

The electronic absorption spectra of the bis acyclic merocyanine dye (3b) and bis cyclic merocyanine dye (5a) in aqueous universal buffer solutions of varying pH values (1.87, 2.51, 3.92, 5.06, 6.89, 8.13, 9.56 and 11.41) showed bathochromic shifts with intensification of their absorption bands at high pH (alkaline media) and



X = C_2H_5 (in ethanol); H (in water)

Scheme 3.

hypsochromic shifts with quenching the intensity of the bands at low pH media (acidic media), Table 3, Fig. 1 (B). So, the mentioned dyes which have an electrons lone pair of on the N-ph pyrazole nitrogen atom undergoes to protonation in low pH (acidic media). This leads to a criterion of positive charge on N-Ph pyrazole nitrogen atom, and consequently the electronic charge transfer pathways to the positively charged carbon atom of the carbonyl group will be difficult resulting in a hypsochromic shift for the absorption band, Scheme 3 (B). On increasing the pH of the media, the absorption bands are intensified and bathochromically shifted due to deprotonation of the N-Ph pyrazole nitrogen atom, and consequently the electronic charge transfer pathways to the positively charged carbon atom of the carbonyl group will be easier and facilitated, Scheme 3 (B).

Several methods have been developed for spectrophotometric determination of the dissociation constants of weak acids. The variation of absorbance with pH can be utilized. On plotting the absorbance at the λ_{max} vs. pH, S-shaped curves are obtained, Fig. 1 (B), Table 3.

In all of the S-shaped curves obtained, the horizontal portion to the left corresponds to the acidic form of the indicator, while the upper portion to the right corresponds to the basic form, since the pK_a is defined as the pH value for which one half of the indicator is in the basic form and the other half in the acidic form. This point is determined by intersection of the curve with a horizontal line midway between left and right segments [32]. The acid dissociation or protonation constants values of the dyes are summarized in Table 3.

2.3. Conclusion

From the results discussed in this study we can conclude that due to the spectral and/or the photosensitization properties of these cyanine dyes, they can be used and/or employed as photographic sensitizers for silver halide emulsions in photographic industry because they can increase the sensitivity range of the photographic emulsions by making an increase in the range of wavelength which form an image on the film. In addition, these cyanine dyes can be used as indicators for solvent polarity due to their solvatochromic properties. Besides, these cyanine dyes can be used as acid–base indicators in analytical chemistry. This is because these dyes have halochromic properties, where they gives a reversible colour change as a result of a change in the pH. Halochromic compounds are acids or bases in which a change in the pH causes a change in the ratio of ionized and non ionized states, as these two states have different colours, the colour of the solution changes (rewords). This colour change can be used in acid-base titrations in analytical chemistry, where the colour change of the halochrome corresponds to the end-point of the reaction.

3. Experimental

3.1. General

All the prepared compounds were purified using chromatographic techniques (Column chromatography). Melting points were measured using Galenkamp melting point apparatus and are uncorrected. Elemental analyses were carried out at the Microanalytical Center at Cairo University by an automatic analyzer (Heraeus). IR (KBr pellets) spectra were determined in 1650 FT-IR instrument (Cairo University), and the ^{1}H NMR spectra were accomplished using 300 MHz NMR Spectrometer (Cairo University). Electronic visible absorption spectra were carried out on Shimadzu UV–Visible recording spectrometer (South Valley University, Faculty of Science at Aswan).

3.2. Synthesis

3.2.1. Synthesis of 3,9-diethyl-4,10-diformyl-2,8-diphenyl-5,11-dihydro-benzo[2,3-b; 2′, 3′ - b′]bis pyrazolo[4,5-b]-l,4-(oxa-, thia-, andpyra-)zine 1a–c

The synthesis of compounds 6a–c was carried out as in the reported procedure [23].

3.2.2. Synthesis of 3,9-diethyt-(l0-formyl)-2,8-diphenyl-5,ll-dihydro-benzo[2,3-b; 2′, 3′ - b′]bis pyrazolo[4,5-b]-l,4-(oxa-, thia-, and pyra-)zine-4-acyclic merocyanine 2a–e

A mixtures of equimolar ratios (0.01 mol) of {acetone, acetylacetone, or ethylacetoacetate} and bis formyl compounds 1a–c were refluxed for 8 h in ethanol (100 ml) as organic solvent containing piperidine (1 ml) as basic catalyst. The reaction mixture, which changed from brown to deep brown during the reaction, was filtered while hot to remove unreacted material, concentrated, cooled, neutralized with glacial acetic acid and precipitated by adding cold water. The acyclic merocyanine dyes 2a–e were dried, collected and crystallized from ethanol. The relevant data are given in Table 1.

3.2.3. Synthesis of 3,9-diethyt-2,8-diphenyl-5,ll-dihydro-benzo[2,3-b; 2′, 3′ - b′]bis pyrazolo[4,5-b]-l,4-(oxa-, thia-, and pyra-)zine-4,10-bis acyclic merocyanine dyes 3ae

Two different routes were used to synthesize these cyanine dyes.

3.2.3.1. *Route (1).* 1 : 2 M ratios of the bis formyl compounds 1a–c and (acetone, acetylacetone, or ethylacetoacetate) were heated under reflux for 8 h in ethanol (100 ml) containing piperidine (1 ml). The reaction mixture, which changed from brown to deep brown during the refluxing conditions was filtered while hot to remove unreacted materials, concentrated, cooled, neutralized with glacial acetic acid and precipitated by adding cold water. The bis acyclic merocyanine dyes 3a–e were dried, collected and crystallized from ethanol. The relevant data are given in Table 1.

3.2.3.2. *Route (2).* Piperidine (1–2 ml) was added to a mixture of equimolar ratios (0.01 mol) of 2a–**c** and (acetone, acetylacetone or ethylacetoacetate} dissolved in ethanol. The reaction mixture was heated under reflux for 8 h and attained a deep brown colour at the end of refluxing. It was filtered while hot, concentrated, cooled, neutralized by glacial acetic acid and precipitated by adding cold water. The precipitated products were filtered of, washed several times with water, dried and crystallized from ethanol to give the compounds obtained through Route (1), characterized by melting points, mixed melting points, same IR and ^{1}H NMR spectra data, Tables 1, 4, Scheme 1.

3.2.4. Synthesis of 3,9-diethyl-10-formyl-2,8-diphenyl-5,11-dihydro-benzo[2,3-b; 2′, 3′ - b′]bis pyrazolo[4,5-b]-l,4-(oxa-, thia-, and pyra-)zine-4-cyclic merocyanine 4a–c

Barbitone (0.01 mol) were heated under reflux with equimolar ratios of the diformyl compound 1a–c in ethanol (100 ml) as organic solvent and piperidine (1 ml) as a basic catalyst for 6 h. At the end of the reflux, the reaction mixture was filtered off on hot, concentrated, neutralized with glacial acetic acid and precipitated by adding ice-water mixture. The separated cyclic merocyanine dyes 4a–c were filtered, washed with water, and crystallized from ethanol. The results are listed in Table 1.

3.2.5. Synthesis of 3,9-diethyl-2,8-diphenyl-5,11-dihydro-benzo [2,3-b; 2′, 3′ - b′]bis pyrazolo[4,5-b]-l,4-(oxa-, thia-, and pyra-) zine-4,10-bis cyclic merocyanine dyes 5a–c

These cyanine dyes were prepared by the following two different routes.

3.2.5.1. Route (1). Barbitone (0.02 mol) and diformyl compounds 1a–c (0.01 mol) were dissolved in ethanol (100 ml), and then piperidine (1 ml) was added. The reaction mixture was refluxed for 6 h. At the end of the reflux, the reaction mixture was filtered off on hot, concentrated, neutralized with glacial acetic acid and precipitated by adding ice-water mixture. The separated bis cyclic merocyanine 5a–c were filtered, washed with water, and crystallized from ethanol. The results are listed in Table 1.

3.2.5.2. Route (2). Equimolar ratios (0.01 mol) of barbitone and the previously prepared cyclic merocyanine dyes 4a–c were dissolved in ethanol (100 ml) containing piperidine (1 ml). The reaction mixture was heated under reflux for 6 h and attained a deep permanent colour at the end of refluxing. It was filtered while hot, concentrated and cooled. The precipitated products obtained after addition of cold water, were filtered of, washed with water, collected and crystallized from ethanol to give the same bis-cyclic merocyanine dyes 5a–c obtained by Route (1), characterized by melting points, mixed melting points, same IR and ^{1}H NMR spectral data, Tables 1, 4, Scheme 1.

3.3. Colour spectrophotometric measurements

Colour spectrophotometric measurements for all the prepared cyanine dyes were examined in 95% ethanol solution. Also, colour spectrophotometric measurements for some selected synthesized cyanine dyes were investigated in pure solvents having different polarities and/or in aqueous universal buffer solutions. The solvents used were spectroscopic grade and purified according to recommended methods [33]. All the spectral data were measured and recorded using 1 cm Qz cell in Shimadzu UV–Visible Recording Spectrophotometer. A stock solution (10-3 M) of the dyes was diluted to a suitable volume in order to obtain the required concentrations.

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