Studies on Solvent Dependence of Absorption Bands of Some Selected Cyanine Dyes

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The solvent dependence of the short-wavelength absorption bands of selected cyanine dyes incorporating 1*H*-pyrazolo[4,3-*d*]-[1,2,3]oxadiazole moiety was studied in the visible region. The spectra show a complex charge transfer CT transition and the assignments are based on solvatochromic behaviour of this transition. The absorption spectra in buffer solution have been also utilized to select the optimal pH value at which they can be applied as photosensitizers.

It is of interest to note that oxadiazole moiety has been investigated in view of its biological [1—3], pesticidal [4, 5], CNS stimulant, anti-inflammatory, and hypotensive action in experiments [6]. The use of such rings as a main entity in the synthesis and electronic spectra of cyanine dyes are yet scarcely mentioned in literature. Such dyes might exhibit photosensitization effect in blue-green light [7] and might be used as inhibitors of the cell growth and division [8]. Thus, the present work describes the solvatochromic behaviour of some selected 1*H*-pyrazolo[4,3-*d*]-[1,2,3]oxadiazole cyanine dyes (*Ia—Ic, IIa, IIb,* see Scheme 1) and their optimal pH value to explore the possibility of the suitable conditions in the application of these dyes as photosensitizers.

spectrophotometer, visible spectra on a Unicam SP 1750 ultraviolet spectrophotometer, and PMR spectra on a 90 MHz EM 390 spectrometer using TMS as internal standard.

The organic solvents used were of spectral grade or purified according to the recommended methods [10, 11]. The electronic absorption spectra of the studied dyes in different organic solvents were recorded within the wavelength range 350—700 nm using the concentration of 10^{-4} mol dm^{-3}. Solutions of low concentrations used in spectral measurements were obtained by accurate dilution.

Aqueous universal buffer solutions of pH 2.40—12.16 were prepared [12]. The pH values of these solutions were checked at 25 °C using an Orion pH-

Ia—Ic a X = CH, R = *p*-OH
b X = CH, R = *p*-N(CH₃)₂
c X=N,   R = *p*-OH

IIa, IIb a *n* = 0
b *n* = 1

Scheme 1
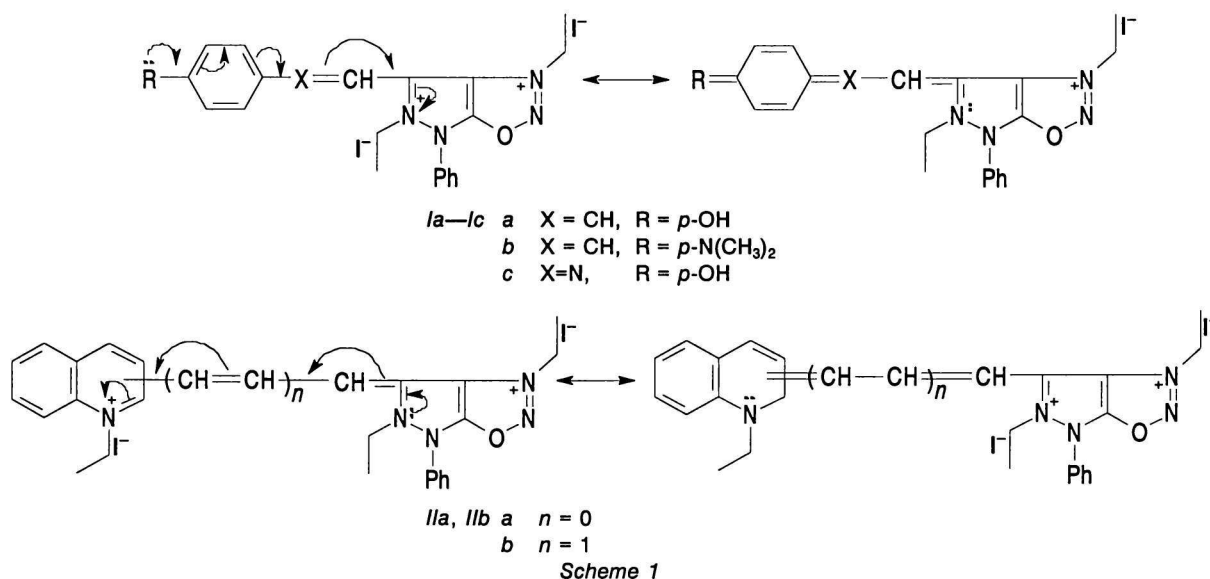
EXPERIMENTAL

Melting points are uncorrected. IR spectra were recorded on a Perkin—Elmer infrared 127B

meter, model 60/A accurate to ± 0.005 pH units. Ethanolic solution (0.5 cm³) containing 1 × 10⁻⁴ mol dm⁻³ of *Ia, Ic,* and *IIa* was added to the buffer solution (4.5 cm³).

1H-Pyrazolo[4,3-d]-[1,2,3]oxadiazole Styryl (Azastyryl)cyanine Dyes Ia—Ic

2,4-Diethyl-3-methyl-1-phenylpyrazolium[4,3-d]-[1,2,3]oxadiazolium diiodide (0.01 mol) [9] was refluxed for 10—15 h with p-hydroxy or p-dimethyl-aminobenzaldehyde and p-nitrosophenol (0.01 mol) in the presence of ethanol (30 cm^3) containing piperidine. The product was cooled, triturated with few drops of acetic acid, followed by ethanol, filtered, washed several times with ethanol and crystallized from aqueous ethanol (Table 1).

[4,3-d]-[1,2,3]oxadiazolium diiodide. Yield = 85 %, m.p. = 172 °C.

For $C_{22}H_{25}N_5O_2I_2$ (M_r = 645) w_i(calc.): 40.93 % C, 3.88 % H, 10.85 % N; w_i(found): 41.03 % C, 3.95 % H, 10.97 % N.

Refluxing of the latter compound and 2-methyl-isoquinolinium salt in AC_2O (20 cm^3) for 2 h and working up in the same way as described for IIa gave compound IIb as trimethine type (Table 1).

The structure and purity of the above compounds were checked by elemental analysis and IR and PMR spectral data (Table 1).

Table 1. Characterization of the Investigated Cyanine Dyes

Compound	Formula M_r	w_i(calc.)/% w_i(found)/% C	H	N	Yield %	M.p. °C	Colour	IR (KBr) λ_{max}/cm^{-1} Assignment	PMR (CDCl$_3$) δ	Assignment
Ia	$C_{21}H_{22}N_4O_2I_2$ 616	41.0 40.9	3.7 3.6	9.2 9.1	52	125	Intense violet	2960 (Ethoiodide) 1600 v(C=C) 1590 v(N=N)	6.2—7.4 3.2 0.95—1.2 2.2	(m, 5H, H$_{arom}$) (d, 2H, olefinic) (t, 6H, 2 × CH$_3$) (q, 4H, 2 × N—CH$_3$)
Ib	$C_{23}H_{27}N_5OI_2$ 643	43.0 42.9	4.4 4.2	11.0 10.9	50	142	Pale violet			
Ic	$C_{20}H_{21}N_5O_2I_2$ 617	39.0 38.9	3.6 3.4	11.4 11.4	45	120	Violet	2950 (Ethoiodide) 1590 v(CH=N)	6.45 3.2 2.1 1.6	(m, 9H, H$_{arom}$) (s, 1H, CH=N) (q, 4H, 2 × CH$_2$) (t, 6H, 2 × N—CH$_3$)
IIa	$C_{25}H_{27}N_5OI_2$ 667	45.1 45.0	4.1 4.1	10.7 10.5	75	107	Violet	2990 (Ethoiodide) 1650 v(C=C) 1590 v(N=N)	6.55—7.2 4.2 2.1—2.75	(m, 11H, H$_{arom}$) (s, 1H, olefinic) (q, 4H, 2 × CH$_2$ + t, 6H 2 × CH$_3$ of ethoiodide)
IIb	$C_{27}H_{29}N_5OI_2$ 693	46.8 46.8	4.3 4.2	10.2 10.2	60	120	Purple	2960 (Ethoiodide) 1620 v(conj. C=C) 1590 v(N=N)		Cited signals mentioned for IIa and δ = 3.8 (t, 3H, conj. olefinic protons)

1H-Pyrazolo[4,3-d]-[1,2,3]oxadiazole-mono(tri)-methine Cyanine Dyes IIa, IIb

To a solution of 2,4-diethyl-3-methyl-1-phenyl-pyrazolium[4,3-d]-[1,2,3]oxadiazolium diiodide (0.01 mol) and 1-ethylquinolinium iodide (0.01 mol) in ethanol (20 cm^3) few drops of piperidine were added. The reaction mixture was refluxed for 3 h, filtered, hot concentrated, cooled and precipitated by dilution with water. The precipitated product was collected, washed with ethanol and then crystallized from aqueous ethanol to give IIa as monomethine type.

2,4-Diethyl-3-methyl-1-phenylpyrazolium[4,3-d]-[1,2,3]oxadiazolium diiodide (0.01 mol) was refluxed with diphenylformamidine (0.25 mol) and acetic anhydride (25 cm^3) for 90 min. The product was collected, washed with ethanol and crystallized from acetic acid to give brown needles as a key intermediate, namely 3-anilinocarbamoylmethyl-2,4-diethyl-(N-phenylcarbamoylmethyl)pyrazolium-

RESULTS AND DISCUSSION

The visible absorption spectra of selected 1H-pyrazolo[4,3-d]-[1,2,3]oxadiazole cyanines Ia—Ic, IIa, and IIb in 95 % ethanol show a longer absorption band in the wavelength range 300—700 nm. This band can be assigned to the π—π* transition of the unsaturated centres within the whole molecule. A considerable charge transfer CT character of this band depends on the type of selected cyanine. Thus, the charge transfer of styryl and azastyryl cyanines Ia, Ib, and IIc characterized by the CT band may take place from 4-aryl substituents (R) as electron donor to the pyrazolo[4,3-d]-[1,2,3]oxadiazol-3-ium salt. On the other hand, it may take place from N-ethyl group of pyrazolo[4,3-d]-[1,2,3]oxadiazole as electron donor to the quinolin-2-ium quaternary salt as electron acceptor centre for mono- and trimethine cyanines IIa and IIb. Such a charge transfer is influenced by the nature of electron-donating group or by the substitution on the methine group exhibiting a bathochromic shift (Table 2).

Table 2. Electronic Absorption Spectra of Some New 1*H*-Pyrazolo[4,3-*d*]-[1,2,3]oxadiazole Cyanines in Organic Solvents

Compound	Ethanol	2-Methylpropanol λ_{max}/nm $(\varepsilon_{max}/(cm^2\ mol^{-1}))$	Chloroform	Dimethylformamide
Ia	360 (19200)	355 (17520)	365 (8520)	365 (17580)
Ib	350 (65600)	340 (32800)	340 (36000)	340 (32000)
	450 (18880)	445 (6400)	440 (8000)	450 (6500)
Ic	360 (11400)	365 (20880)	365 (12920)	355 (6600)
	440 (6400)	445 (9420)	455 (13400)	525 (2080)
IIa	440 (4100)	440 (16500)	440 (8600)	440 (8440)
	520 (6600)	–	–	530 (6760)
IIb	455 (4100)	507 (4800)	482 (4260)	440 (4000)
	485 (4440)	575 (4700)	–	515 (4160)
	555 (4200)	–	–	565 (4000)

The charge transfer in *Ia*—*Ic* is considered as an intermolecular CT transition within the whole molecule. This is based on the fact that the high electron-withdrawing character of 1*H*-pyrazolo[4,3-*d*]-[1,2,3]oxadiazol-3-ium salt as well as the high electron-releasing character of substituents on aryl, e.g. *p*-OH and *p*-N(CH₃)₂, causes a forced planarity of the molecule. In the case of *IIa* and *IIb* the intermolecular CT transition is based on the high electron-withdrawing character of quinolium cation as well as increasing of conjugation of the side chain (CH=CH)ₙ or releasing of electrons from nitrogen atom bearing an ethyl group of 1*H*-pyrazolo[4,3-*d*]-[1,2,3]oxadiazole. From Table 2 it is clear that the unexpected high excitation energy of CT band in compounds *Ia*—*Ic* is attributed to the antagonizing effect of the phenyl group attached to 1*H*-pyrazolo-[4,3-*d*]-[1,2,3]oxadiazoles, meanwhile this band acquires a lower excitation energy than the conjugation of the side chain.

Effect of Solvents

The electron absorption of the styryl cyanines *Ia*—*Ic*, mono- and trimethine *IIa* and *IIb* in the wavelength range 300—700 nm is studied in different organic solvents, *viz.* isobutanol, chloroform, and dimethylformamide. This was made with the intention to investigate the solvatochromic behaviour of these dyes (λ_{max} and ε_{max} values of the intermolecular charge transfer CT bands are given in Table 2). It is clear from these data that the λ_{max} of the intermolecular CT band exhibits a bathochromic shift on transfer from ethanol to dimethylformamide in compounds *Ia*—*Ic*, *IIa*, and *IIb* as a result of the increase in solvent polarity. Also, the bands corresponding to *n*—π* and CT transitions show a slight red shift on changing the solvent from ethanol to chloroform in compounds *Ia* and *Ic*. This may be explained as a result of H-bond formation between ethanol and the lone electron pair of OH group. This decreases slightly the electron density on OH group and consequently decreases to some extent the mobility of the attached π-electrons to the conjugated pathway.

The extra band observed at longer wavelength in the spectra of compound *IIa* (λ_{max} (ethanol) = 520 nm, λ_{max} (dimethylformamide) = 530 nm) and compound *IIb* (λ_{max} (ethanol) = 555 nm, λ_{max} (dimethylformamide) = 565 nm) relative to chloroform can be ascribed to the formation of hydrogen bonding which plays an important role. The band observed is presumably due to the intermolecular CT transition. This involves an electron transfer of the lone electron pair of the nitrogen atom of 1*H*-pyrazolo[4,3-*d*]-[1,2,3]-oxadiazole ring of the solute molecule to the antibonding orbital of OH and H—C=O bonds belonging to ethanol and dimethylformamide solvents, respectively. Furthermore, the possibility of formation of a H-bonded solvated complex with ethanol, dimethylformamide molecules rather than chloroform molecules is presumably due to the low ionization potential of the former solvents than of the latter one. This in turn results in an easier formation of H-bonded solvated complex with ethanol and dimethylformamide molecules (Fig. 1a—c).

Spectra in the Mixed Organic Solvents

The visible spectra of compounds *Ic* and *IIa* were investigated in the CHCl₃—DMF mixture in order to study the H-bonded solvated complex liable to be formed between the solute molecules and the H-bond acceptor solvents.

The visible spectrum of the compound *Ic* in pure CHCl₃ displayed only one band at λ_{max} = 440 nm (*cf.* Fig. 2a). On adding DMF a new band at λ_{max} = 530 nm appeared while its absorbance increased with the increasing molarity of DMF and the absorbance of the band at λ_{max} = 440 nm decreased. A fine isosbestic point was obtained in this case indicating the existence of an equilibrium between the solvated complex and the free solute molecules (Fig. 2a).

Similarly, the visible spectrum of *IIa* in the CHCl₃—DMF mixture showed absorption band at λ_{max} = 440
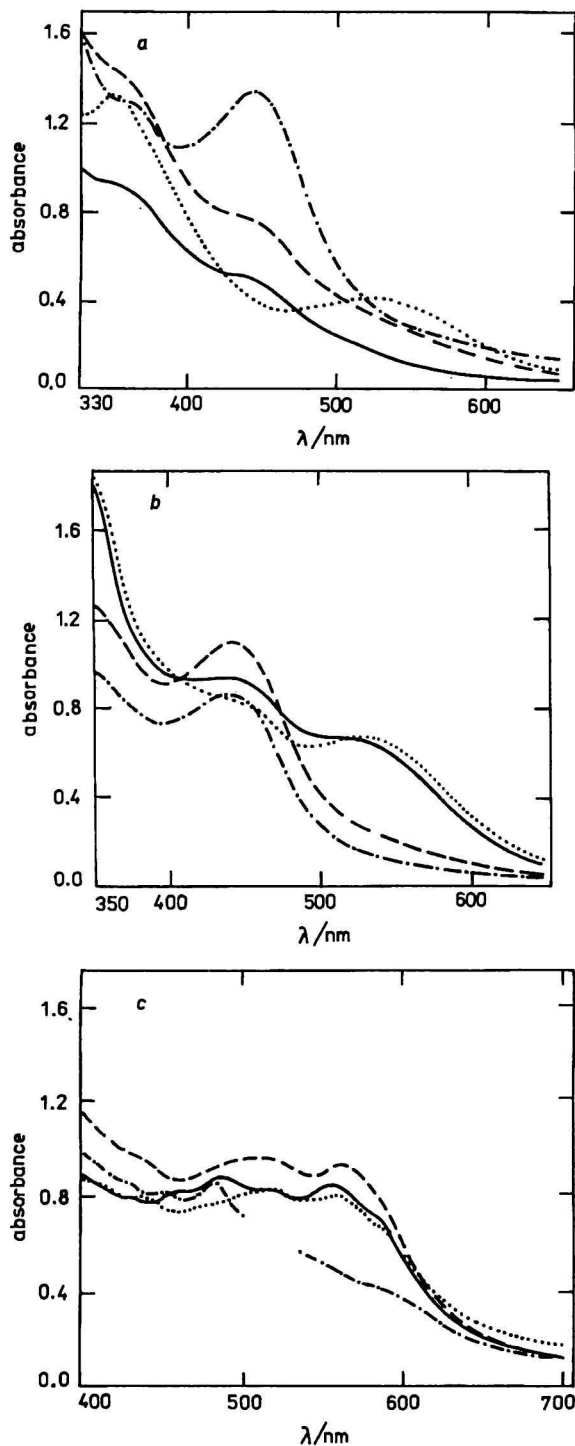
Fig. 1. *a*) Electronic absorption spectra of *Ic* in ethanol (——), DMF (····), CHCl$_3$ (— · —), and 2-methylpropanol (— —). *b*) Electronic absorption spectra of *IIa* in ethanol (——), DMF (····), CHCl$_3$ (— · —), and 2-methylpropanol (— —). *c*) Electronic absorption spectra of *IIb* in ethanol (——), DMF (····), CHCl$_3$ (— · —), and 2-methylpropanol (— —).

nm in pure CHCl$_3$ and on adding DMF a new band at $\lambda_{max} = 525$ nm appeared at the same time while the absorbance of the shorter-wavelength band decreased and a fine isosbestic point was obtained.
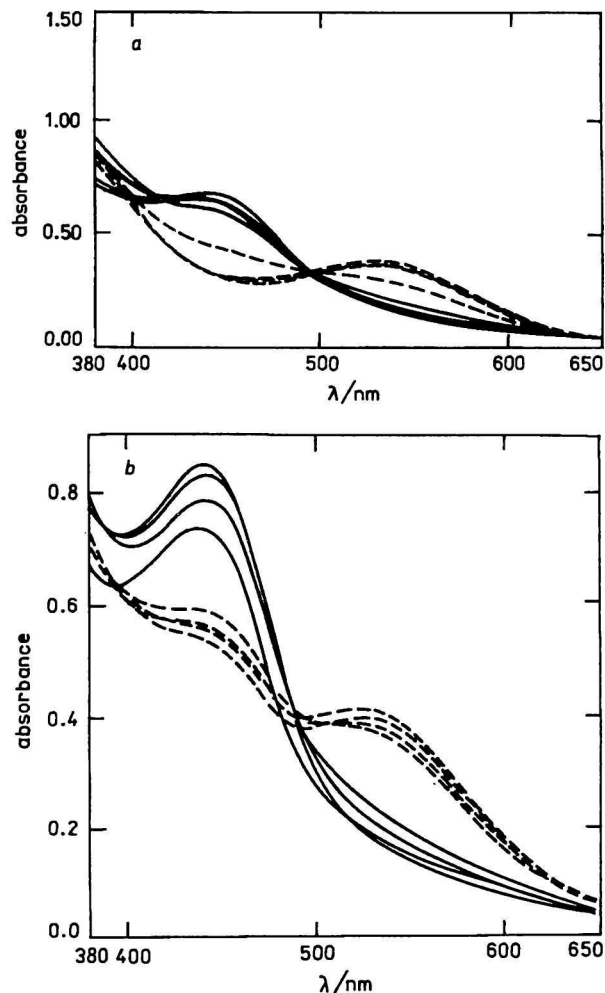
Fig. 2. *a*) Electronic absorption spectra of *Ic* ($c = 1 \times 10^{-4}$ mol dm^{-3}) in the CHCl$_3$ —DMF mixture. *b*) Electronic absorption spectra of *IIa* ($c = 1 \times 10^{-4}$ mol dm^{-3}) in the CHCl$_3$—DMF mixture.

This behaviour indicates that DMF molecules have greater tendency to form solvated complex with the solute molecules relative to CHCl$_3$. This is due to the low ionization potential of DMF as well as its high H-bond accepting character (Fig. 2*b*).

Convincing evidence for the CT nature of this band is provided by studying the spectral behaviour of *Ia*, X = CH, *Ic*, X = N, and *IIa* in aqueous universal buffer solution of varying pH (Fig. 3*a*—*c*).

This is performed in order to ensure the optimal pH in the application of these dyes as photosensitizers. The effectiveness of the compounds as photosensitizers increases when they are present in the ionic form which has a higher planarity. Thus, the electronic absorption spectra of compounds *Ia* and *Ic* in aqueous universal buffer solutions (Fig. 3*a*, *b*) showed that the absorption bands of the compounds *Ia* and *Ic* are largely red shifted (in alkaline media). This large red shift is mainly due to a relatively increased charge density on OH group attached to aryl moiety. On the other hand, this band
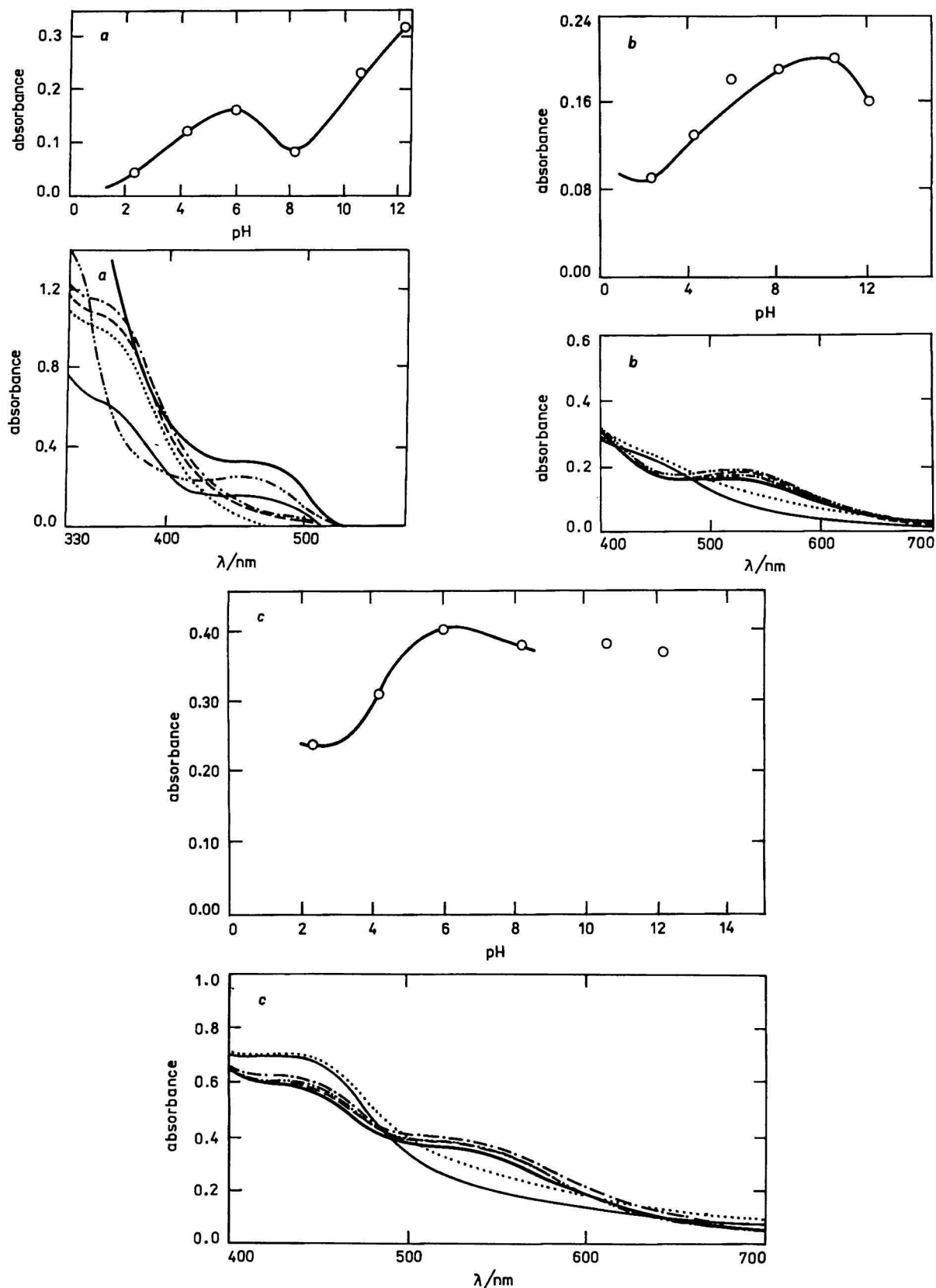
Fig. 3. a) Electronic absorption spectra of *Ia* in aqueous universal buffer solutions at pH 2.30 (···), 4.24 (— · —), 5.91 (— —), 8.17 (——), 10.60 (— ·· —), and 12.16 (——). λ_{max} = 460 nm, c = 1 × 10^{-4} mol dm^{-3}, pK_a = 7.2. b) Electronic absorption spectra of *Ic* in aqueous universal buffer solutions at pH 2.30 (——), 4.24 (···), 5.91 (— —), 8.17 (— —), 10.60 (— ·· —), and 12.16 (——). λ_{max} = 530 nm, c = 1 × 10^{-4} mol dm^{-3}, pK_a = 6. c) Electronic absorption spectra of *IIa* in aqueous universal buffer solutions at pH 2.30 (——), 4.24 (···), 5.91 (— · —), 8.17 (— —), 10.60 (— ·· —), and 12.16 (——). λ_{max} = 530 nm, c = 1 × 10^{-4} mol dm^{-3}, pK_a = 4.5.

is blue shifted (in low pH acidic media). This can be interpreted on the principle that OH group becomes protonated in solutions of low pH and therefore the CT interaction within the protonation form is expected to be difficult, *i.e.* the protonated form does not absorb in the visible region. Meanwhile, as the pH of the media increases, the OH group becomes deprotonated and therefore its mesomeric interaction with the rest of molecule becomes high, consequently the CT interaction within the free base is facilitated, *i.e.* the free base absorbs in the visible region.

Similarly the electronic absorption of compound *IIa* in universal buffers showed absorption bands, their positions are influenced by the pH values, *i.e.* it has been found that these bands are largely red shifted in alkaline media and blue shifted in acidic media. Thus, nitrogen atom bearing ethyl group of 1*H*-pyrazolo[4,3-*d*]-[1,2,3]oxadiazole moiety in compound *IIa* becomes protonated in solution of low pH and on the other hand, as the pH of the media increases, the nitrogen atom bearing this group becomes deprotonated (Fig. 3*c*).

By plotting the absorbance at λ_{max} *vs.* pH, S-shaped curves were obtained. The values are listed in Table 3. The horizontal portion to the left of S-curves corresponds to the acidic form of the compound, while the upper portion to the right corresponds to the basic form. Therefore the pK_a is defined as the pH value for which one half of the compound 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 the left and right segments. From Fig. 3*a* the pK_a value for compound *Ia* is 7.2, from Fig. 3*b* the pK_a value for compound *Ic* is 6, and from Fig. 3*c* the pK_a value for compound *IIa* is 4.5.

Table 3. Electronic Absorption Spectra of *Ia, Ic,* and *IIa* in Aqueous Universal Buffer Solutions

pH	Absorbance		
	λ_{max} = 460 nm *Ia*	λ_{max} = 530 nm *Ic*	λ_{max} = 530 nm *IIa*
2.30	0.04	0.09	0.24
4.24	0.12	0.18	0.31
5.91	0.16	0.18	0.40
8.17	0.08	0.19	0.38
10.60	0.24	0.20	0.38
12.16	0.32	0.16	0.36

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