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Synthesis and spectroscopic characterisation of heptamethincyanine NIR dyes for their use in optochemical sensors

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Abstract

A set of near-infrared absorbing tricarbocyanine dyes has been synthesised and characterised for their future application as chromoionophores in optochemical sensors. Their absorption maxima are localised in the NIR region, where matrix interferences are minimal and the use of suitable and inexpensive optical communication components gives great advantages. The acid form of the synthesised dyes in pure ethanol spans the region from 675 to 815 nm and the molar absorptivities are up to 3.3×10^5 l/mol cm. The calculated pK_a values in ethanol lie between 11.4 and more than 13. The effect of the introduction of substituents on the pK_a values and on the spectroscopic characteristics of the dyes is also discussed. Moreover, pH-sensitive aggregation processes have been observed in aqueous solution.

Keywords: Heptamethincyanine dyes; NIR; LED laser; pH-indicators

1. Introduction

Few years ago, a type of plasticized PVC optodes based on the well known ion-selective electrodes was proposed for optical sensors [1,2]. These selective membranes should contain a chromoionophore, which could provide the optical transduction of the signal generated by the chemical interaction. Since direct and selective analyte–chromoionophore interactions were usually difficult to attain or were irreversible, a combination of a certain selective ionophore and a chromophore was proposed to be added to the membrane composition. The analyte–ionophore interaction alters in some way the spectroscopic characteristics of the chromophore in order to maintain the electroneutrality of the membrane. In the case of a pH indicator, the chromophoric structure is modified when a proton is released or added when the ionic analyte penetrates into the membrane.

Classic acid–base indicators, with absorption maxima in the visible region of the electromagnetic spectrum, are widely used in aqueous acid–base titrations. They would be potential chromoionophores in the visible region but their solubility in the commonly used plasticizers for PVC membranes is limited.

On the other hand, the recent outgrowth of the telecommunication industry has provided the technological support needed to accelerate the development of optochemical sensors since new and cheap, active and

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passive optical components have been developed. In order to take profit of such advantages, new chromophores with acid–base properties are needed. Their absorption bands should match the wavelengths of the cheap light sources, which are employed in the telecommunication industry, such as the semiconductor laser diodes (LED laser), which present an intense monochromatic light emission in the NIR region. The NIR spectral region is clean of chemical interferences since only scarce groups of molecules are spectroscopically active and hence, a considerable specificity can be achieved [3,4]. Actually, some dyes with absorption bands in the NIR region are commercially available but most of them lack solubility in plasticized PVC membranes.

Different cyanine dyes have been synthesised in the research group. A basic polymethinic structure with five vinylic groups, which exhibits an absorption maximum very close to that of the 780 nm LED laser [5], has been chosen. The structure of the indicators is similar to other previously described dyes [6,7], but new groups and substituents have been introduced in order to slightly modify the wavelength of the absorption maxima and the acid–base properties. Finally, substitution of the nitrogen of the heterocycles has been done with the intention of providing solubility to the dye in an organic medium.

The synthesised dyes have been characterised in ethanolic solution. All of them present a sharp absorption band in the NIR region, show a significant acid–base response and their basicity slightly depends on the groups attached to the general structure.

2. Results and discussion

2.1. Synthesis of chromoionophores *3*

Symmetrical chloro dyes (**3**) (Table 1) have been synthesised by refluxing a solution with two equivalents of quaternary salts of heterocyclic bases (**1**) containing an activated methyl group and one equivalent of a pentamethine salt (**2**) in absolute ethanol with anhydrous sodium acetate by the procedure of Makin et al. [8] (Scheme 1). The reported yields are calculated for pure isolated compounds. The symmetry of the molecule makes easier the synthesis as well as the purification of the dyes because other secondary products are avoided.

2.2. Synthesis of chromoionophores *4*

In some cases, the vinylic chlorine of the symmetrical dyes has been further substituted by nucleophilic groups, such as 4-aminothiophenol and piperidine. With this method we have obtained four new dyes (Table 2). The synthesis is performed according to a reported procedure [9,10] (Scheme 2).

2.3. Acid–base behaviour in alcoholic solution

The spectroscopic properties of the cyanine dyes have been studied in ethanol since it is known that they are soluble over an extended concentration range. We have determined the maximum absorption wavelength and the molar absorptivity of the acid and the basic forms (Table 3). In all cases, the acid form shows a sharp absorption band in the NIR region, near 780 nm and presents high molar absorptivities (8×10^4–3×10^5 l/mol cm). The basic form appears in the VIS region and has always a lower intensity than the acidic one.

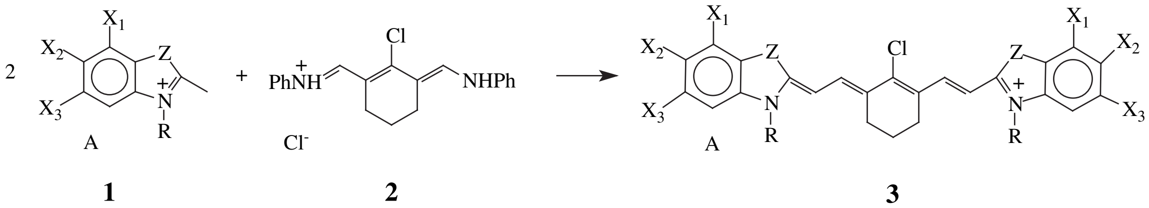
As the hydrogen atoms from the polymeric chain are substituted by electron-releasing or electron-donating compounds [5], some changes in the maximum absorbance wavelength of the cyanine dyes are predicted. The observed bands of dyes **4**, which contain a 4-aminophenylthio group in *meso*-position, present moderate bathochromic shifts from their relative compounds of **3**. Dye **4a**, which is substituted by the piperidine group,

Table 1
Structure and yield of the synthesised cyanine dyes **3**

Dye	X_1	X_2	X_3	Z	R	A	Yield (%)
3a	H	H	H	$C(CH_3)_2$	C_2H_5	I^-	81
3b	H	COOH	H	$C(CH_3)_2$	C_2H_5	I^-	41
3c	H	OH	H	$C(CH_3)_2$	C_2H_5	I^-	36
3d	OH	COOH	H	$C(CH_3)_2$	C_2H_5	I^-	42
3e	H	CH_3CONH	H	$C(CH_3)_2$	C_2H_5	I^-	47
3f[a]	H	H	OH	$C(CH_3)_2$	C_2H_5	I^-	61
3g	H	H	H	S	C_2H_5	I^-	65
3h	H	H	H	S	$C_4H_8SO_3^-$	Na^+	94
3i[b]	H	NH_2	H	$C(CH_3)_2$	C_2H_5	I^-	24

[a] From mixture of isomers.

[b] Obtained from **3e**.



Scheme 1. Reaction scheme for the synthesis of cyanine dyes **3**.

shows a pronounced hypsochromic shift due to the strong electron-donating properties of the substituent.

The nature of the terminating heterocycle is another factor that affects the wavelengths of the absorption maxima of the chromoionophores [11,12]. Two different kinds of heterocycles have been used: benzothiazole and indolenine. We have observed a larger shift to the infrared for the benzothiazole-based dyes than for the indolenine-based dyes as it was expected for a more basic heterocycle.

The introduction of substituents in the benzene ring has to be considered as well. Most substituents lead to shifts to the infrared, although electron-donating groups usually give the greatest bathochromic shifts [11]. The synthesised compounds present in all cases a small bathochromic shift in the absorption band, whose extent depends on the substituent and its relative position.

The acid–base behaviour of the dyes has also been studied in ethanol. Typical absorption spectra and pH titration plots are given in Fig. 1(a, b).

As shown in Fig. 1(a), the only band that appears between 400 and 1000 nm at neutral pH values is located in the NIR region. Although increasing pH, this band keeps its intensity until a very basic pH is reached. From this value, very small variations resulted in big changes in the absorption spectrum and a band corresponding to the basic form arises around 400 nm. Spectral changes of such kind can be attributed to the addition of a hydroxyl ion to an electrophilic site of the dye structure [13]. This reaction interrupts the conjugation system and gives a hypsochromic shift. The proposed acid–base equilibrium is shown in Scheme 3.

2.4. pK_a values

pK_a values of indicators are important parameters to determine in optochemical membranes as they will modulate the response of chromoionophore–ionophore based recognition systems [14]. The calculation of pK_a in ethanol serves to identify the possible acid–base equilibrium and as an orientation for the expected values in the organic solvent, which will be employed as the plasticizer, because they are usually more acidic in ethanol. For the synthesised cyanine dyes, pK_a values have been first evaluated by monitoring the absorbance maxima located in the NIR region in ethanolic solution. Results are given in Table 3. For all dyes, the pK_a values lie between 11.4 and more than 13. This is consistent with the acid–base mechanism in which the conjugation length is shortened only at high hydroxyl ion concentration (Scheme 3).

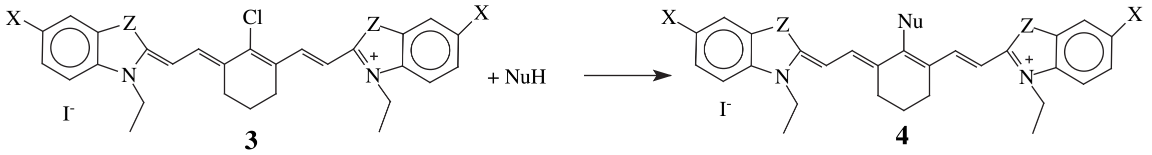
Hence, we can state that the general structure of the synthesised cyanine dyes is responsible for its acid–base equilibrium, while the introduction of substituents can only slightly tailor the spectroscopic properties such as the wavelength of the absorption maxima and the molar absorptivities.

When the substituents attached to the general structure are not pH-sensitive groups (dyes **3a**, **3g**, **3h**, **4a**, **4b**, **4d**), changes in the absorbance spectrum are observed only at high pH values. However, if pH-sensitive substituents are present (dyes **3b**, **3c**, **3d**, **3e**, **3f**, **3i**, **4c**), other bands appear at different wavelengths. These new bands can be related to the changes in the electron properties of the substituents as they change from the ionised to the non-ionised form. Dyes **3c**, **3f** and **3i** show bathochromic shifts at basic pH as well (Fig. 2(a–c)). This fact can be explained in terms of the electronic perturbation produced by the ionisation of substituents. The electron-donating effect of –OH and $-NH_3^+$ groups is strongly increased due to their deprotonation, resulting in a considerable shift to the infrared. For the same dyes, a shift of the pK_a to higher values have been observed as a result of the electron intensity increase in the polymethinic chain, which impedes the nucleophilic addition of the hydroxyl anion.

It is well known that cyanine dyes can aggregate, even in much diluted solutions. It directly depends on the dye concentration [15,16]. The aggregates absorb at different wavelengths from those of the monomer, and consequently, the NIR bands decrease. The aggregation processes can be greatly influenced by the pH of the

Table 2
Structure and yield of the synthesised cyanine dyes **4**

Dye	Initial dye	X	Z	Nu	Yield (%)
4a	**3a**	H	$C(CH_3)_2$	NC_5H_{10}	47
4b	**3a**	H	$C(CH_3)_2$	$SPhNH_2$	46
4c	**3b**	COOH	$C(CH_3)_2$	$SPhNH_2$	42
4d	**3g**	H	S	$SPhNH_2$	45



Scheme 2. Reaction scheme for the synthesis of cyanine dyes **4**.

solution, especially if the structure of the examined dye contains ionisable substituents. Thus, while monitoring the NIR absorbance, we can determine some apparent pK_a values different to the real ones, which reveal aggregation equilibrium. Therefore, we have examined the acid–base behaviour of some of these dyes in aqueous solution. Dye **3b**, for instance, shows aggregate bands in aqueous medium. Following the absorbance maximum of the acidic form, the titration curves give apparent pK_a values at 6.8 and 12.2 for a 2.2×10^{-6} mol/l dye concentration (Fig. 3), when a combined buffer containing 3% v/v methanol is used. But we observe only one pK_a value at 11.4 in pure ethanol (Fig. 4). In Fig. 3(a), it can be seen that aggregation bands appear at lower pH values approximately at 900 nm. Its intensity increases as the monomeric band located at 800 nm decreases. Therefore, these absorbance changes at 800 nm, and the pK_a value of 6.8, cannot be ascribable to perturbations in the π system and so, we can conclude that they are due to dye aggregation processes.

Since aggregation is disfavoured in pure alcohol, no changes are observed for dye **3b** at low pH values (Fig. 4). In this solvent, there is only one pK_a value. Under extreme basic conditions, in the alcoholic and in the aqueous media, a peak with an absorbance maximum located at 425 nm appears, and it intensifies with increasing pH. This new band can be related again to the proposed mechanism for the interaction of the hydroxyl anion with the dye molecule and the subsequent perturbation of the π system (Scheme 3).

Table 3
Wavelengths of the absorbance maximum (nm), molar absorptivities (l/mol cm), and pK_a values determined in ethanol for dyes **3** and **4**

Dye	λ_{acid}	ε_{acid}	λ_{basic}	ε_{basic}	pK_a
3a	780.5	2.97×10^5	421.0	4.11×10^4	11.7
3b	793.5	3.32×10^5	425.5	5.31×10^4	11.4
3c	813.0	2.48×10^5	440.0	1.46×10^5	>13
3d	790.0	2.65×10^5	424.5	5.65×10^4	12.3
3e	814.5	2.51×10^5	426.5	6.36×10^4	12.1
3f	795.0	2.70×10^5	400.0	1.74×10^5	>13
3g	799.0	2.10×10^5	481.5	5.31×10^4	12.6
3h	797.5	2.37×10^5	422.0	8.99×10^4	12.3
3i	782.0	9.29×10^4	425.0	7.81×10^4	>13
4a	678.0	8.47×10^4	527.5	4.42×10^4	13.8
4b	791.5	2.40×10^5	431.0	3.65×10^4	12.4
4c	807.0	2.44×10^5	437.0	4.72×10^4	12.3
4d	807.0	2.24×10^5	490.5	4.80×10^4	13.2

Patonay and co-workers studied the acid–base behaviour of dye **3b**. They reported pK_a values between 7 and 10 for a 4×10^{-6} mol/l solution of the dye in water (0.05% methanol) [6], and between 10 and 13 for the same dye immobilised in a Nafion film [17]. It seems that the changes observed by Patonay between 7 and 10 were caused by the aggregation processes and not by perturbations of the π system of the monomeric dye due to the introduction of substituents, as carboxy groups, in the heterocyclic nuclei. For this reason, when the dye is immobilised in the Nafion film, the aggregation processes are minimised and changes in the bands occur only at higher pH values because of the interaction of the dye with the hydroxyl ion.

The reversibility of the acid–base equilibrium, as the pH is switched back and forth, has also been evaluated by monitoring the absorbance value at the maximum wavelength. In general, extreme alkaline pH values are necessary in order to observe changes in the absorbance, which could decompose the dyes that are usually not fully reversible. This feature makes difficult their implementation in chemically selective optode membranes. Nevertheless, the pK_a can be modified through the immobilisation of the dye in the membrane and by the nature of the plasticizer and the ionophore. Therefore, further experiments of the chromoionophores in membrane are needed to appraise their applicability in optical sensing.

3. Conclusions

A series of 13 cyanine dyes have been synthesised. The wavelengths of the absorbance maximum are located in the NIR region, near the emission of the low cost 780 nm LED laser. Their acid–base equilibrium at high pH values is consistent with the reaction of a hydroxyl ion with an electrophilic site in the structure of the dye. This reaction results in a new absorption band near 400 nm. The dyes present high pK_a values between 11.4 and 13. Further work is in progress for the implementation of the dyes in plasticized PVC membranes as well as for the development of new types of chromoionophores with a similar structure but based on a different acid–base equilibrium that can allow working near neutral pH values.

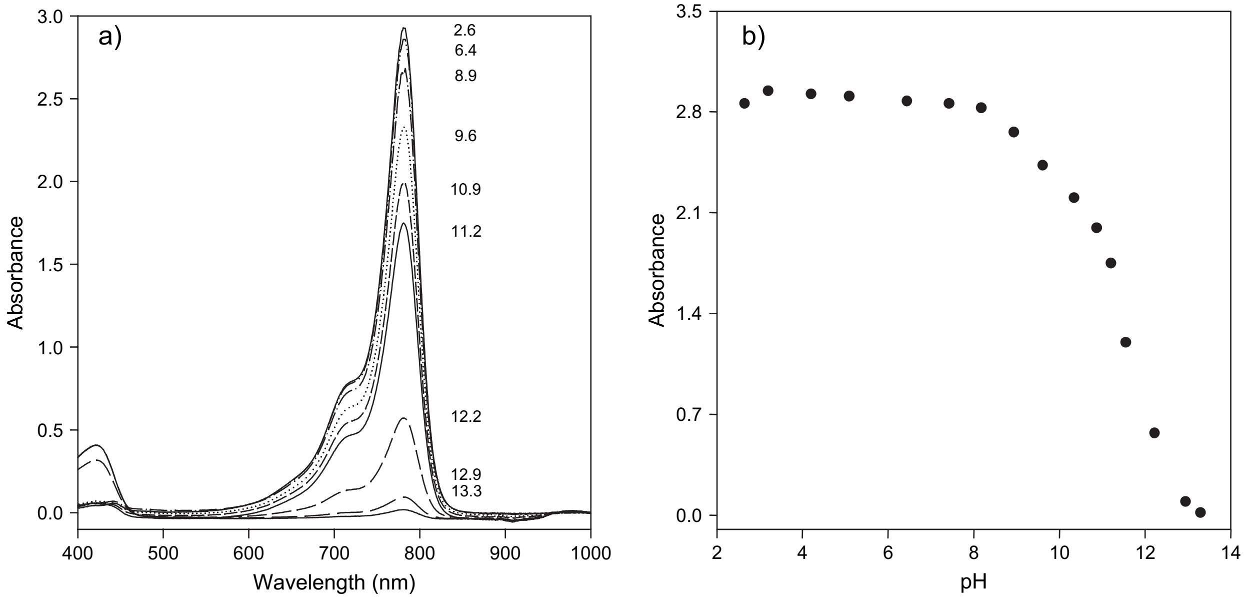


Fig. 1. (a) Absorption spectra for dye **3a** 9.9 × 10^{-6} mol/l in ethanol at different pH values. (b) Titration plot for dye **3a** at 780.5 nm.

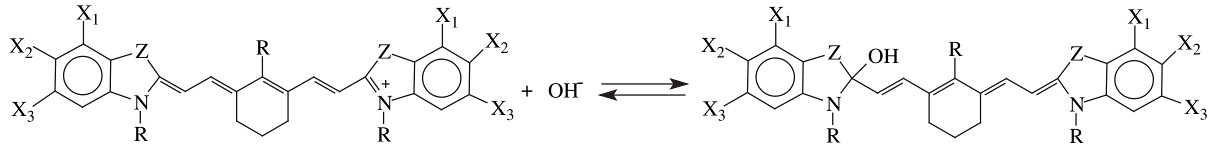
4. Experimental

4.1. General (apparatus and procedures)

All starting materials for the dye synthesis have been purchased form Aldrich Chemical Co. FABMS measurements have been made in a Hewlett Packard HP-5988A. ^{1}H NMR spectra have been measured in a Bruker AC250 at 250 MHz with TMS as internal standard. Reproducibility problems have been found in the elemental analysis due to the hard combustion of this kind of compounds. Spectrophotometric studies have been performed in a UV–VIS–NIR Scanning Spectrophotometer Shimadzu UV-3101PC. pH values have been determined with a pH-meter (Crison-micro pH 2002). The absorption spectra have been recorded between 1000 and 400 nm. The concentration of dye solutions is 1 × 10^{-5} mol/l (unless otherwise indicated), in methanol 99.8% for its characterisation and in ethanol 96% for the spectroscopic evaluation. The characterisation in aqueous solution has been performed in combined buffer solutions (2.85 × 10^{-3} mol/l disodium tetra-borate 10-hydrate, 10 × 10^{-3} mol/l trisodium citrate anhydrous, 6.7 × 10^{-3} mol/l trisodium phosphate 12-hydrate). The pH has been adjusted with HCl and NaOH concentrated solutions. pK_a values have been determined from the calibration curves by the method of the second derivatives.

4.2. Preparation of intermediate compounds

Salts **1a**, **1g** and **1h** are synthesised by quaternisation of the commercially available 2,3,3-trimethylindolenine and 2-methylbenzothiazole, following conventional procedures. Salt **1b** is obtained from the corresponding indolenine, previously synthesised by Fisher cyclisation of hydrazinobenzoic acid and 3-methyl-2-butanone in acetic acid [18]. 5-Hydroxy-2,3,3-trimethylindolenine is obtained by demethylation of the corresponding 5-methoxy derivative by hydrogen bromide. Salt **1d** is obtained from 4-carboethoxy-3-hydroxyphenylhydrazone and 3-methyl-2-butanone with further basic hydrolysis of the ethylester and subsequent quaternisation of the free base. Cyclisation of 4-carboxy-3-hydroxyphenylhydrazone with 3-methyl-2-butanone at the same conditions yields a mixture of 4-hydroxy and 6-hydroxy



Scheme 3. The proposed acid–base equilibrium for dyes **3** and **4** [13].

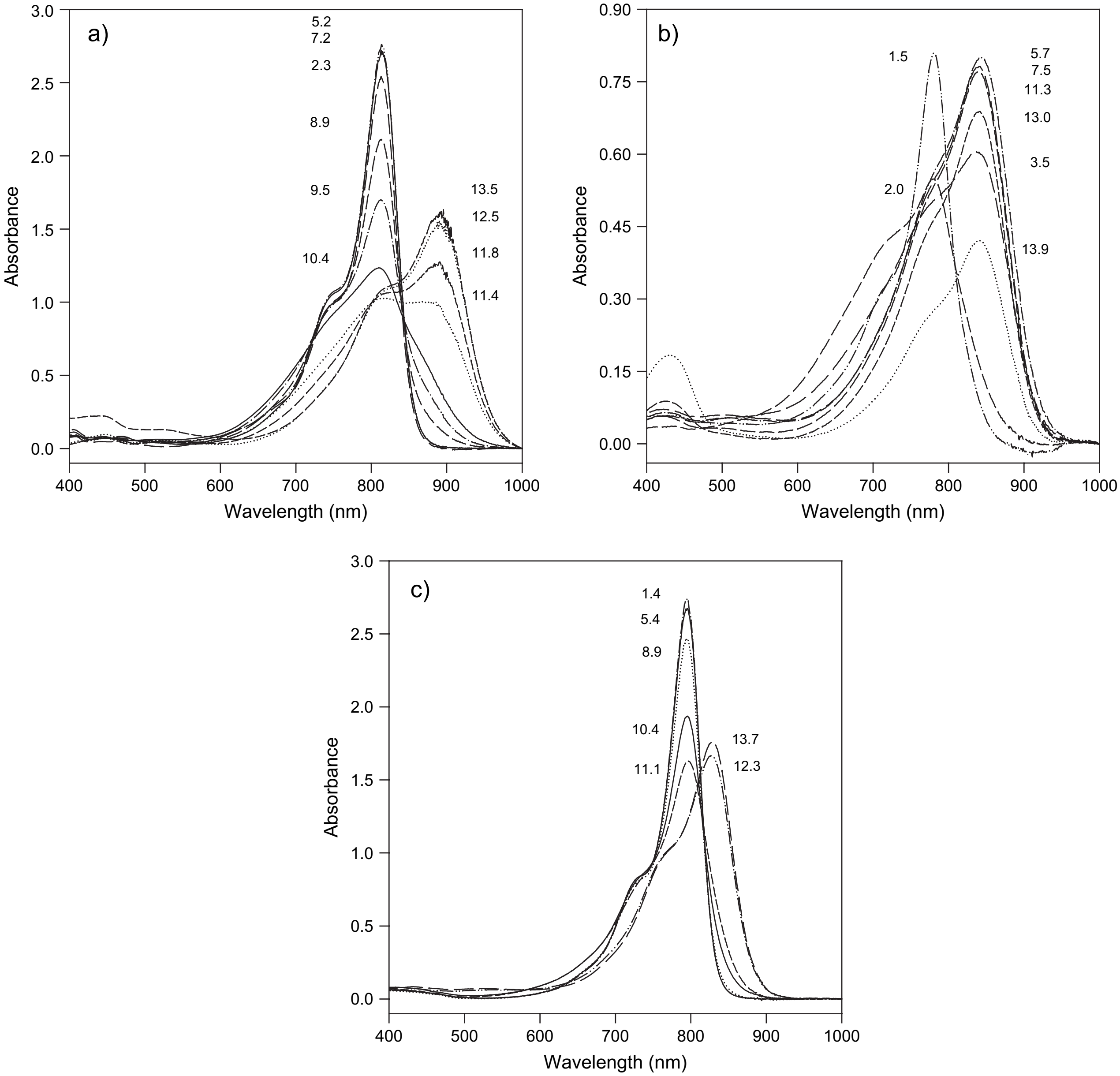


Fig. 2. (a) Absorption spectra of dye **3c** 1.1×10^{-5} mol/l in ethanol at different pH values. (b) Absorption spectra of dye **3i** 1.0×10^{-5} mol/l in ethanol at different pH values. (c) Absorption spectra of dye **3f** 1.0×10^{-5} mol/l in ethanol at different pH values.

indolenines due to the elimination of the carboxyl-group. Attempts for the separation of this mixture by repeated recrystallisations from a variety of solvents (as well as the mixture of their quaternary salts with EtI) do not allow the isolation of any individual isomer. The mixture of the salts is used for the further synthesis of **3f** as follows: 5-acetylamino-2,3,3-trimethyliondolenine is obtained by reduction of 5-nitro-2,3,3-trimethylindolenine [19] with further acetylation by acetic anhydride. Quaternisation of the latter with ethyl iodide gives salt **3e**. The penthamethinecyanine salt (**2**) is prepared from cyclohexanone following the method described by Makin et al. [8].

*4.3. Synthesis of meso-chloro dyes (**3a–h**) [8]*

*4.3.1. Representative procedure for dyes **3***

Two millimoles of the corresponding salt **1**, 1 mmol of **2** and 200 mg of anhydrous sodium acetate in 30 ml of absolute ethanol are heated under reflux for 30 min. After cooling overnight at −10 °C the crystals are filtered out, washed with water, with a little amount of cold ethanol and plenty diethylether.

*4.3.1.1. Dye **3a**.* Yield 520 mg (81%). λ_{max} (MeOH) 788.5 nm ($\varepsilon = 2.27 \times 10^{5}$ l/mol cm). FABMS 511.2 (100%). ^{1}H NMR ($CDCl_3$) δ (ppm) 1.47 (t, $J = 7.3$ Hz,

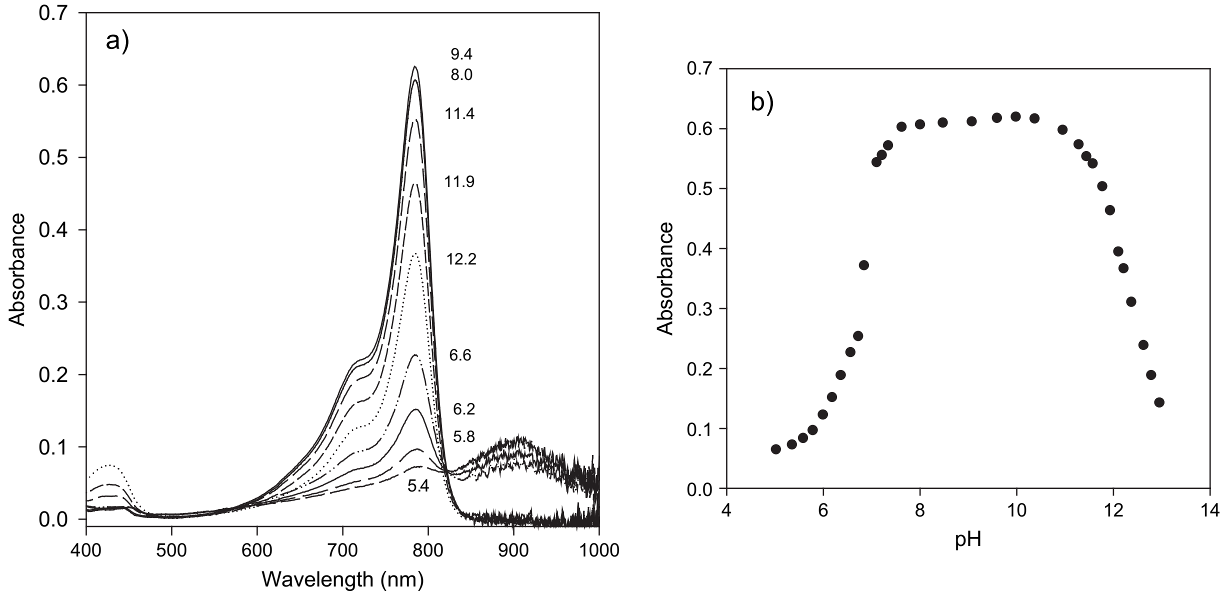


Fig. 3. (a) Absorption spectra of dye **3b** 2.2×10^{-6} mol/l in a combined buffer containing 3% v/v methanol at different pH values. (b) Calibration curve of dye **3b** at 784.0 nm.

6H), 1.73 (s, 12H), 2.02 (quint., $J = 6.6$ Hz, 2H), 2.76 (t, $J = 6.6$ Hz, 4H), 4.27(q, $J = 7.3$ Hz, 4H), 6.22 (d, $J = 14.0$ Hz, 2H), 7.16–7.46 (m, 8H), 8.36 (d, $J =$ 14.0 Hz, 2H).

4.3.1.2. Dye ***3b****.* Yield 450 mg (41%). λ_{max} (MeOH) 789.0 nm ($\varepsilon = 3.14 \times 10^5$ l/mol cm). FABMS 599 (100%). ^{1}H NMR (CD_3OD) δ (ppm) 1.43 (t, $J = 7.2$ Hz, 6H), 1.77 (s, 12H), 1.98 (quint., $J = 5.0$ Hz, 2H), 2.77 (t, $J = 5.0$ Hz, 4H), 4.26 (q, $J = 7.2$ Hz, 4H), 6.38 (d, $J = 13.3$ Hz, 2H), 7.39 (d, $J = 8.3$ Hz, 2H), 8.10–8.13 (m, 4H), 8.49 (d, $J = 13.3$ Hz, 2H).

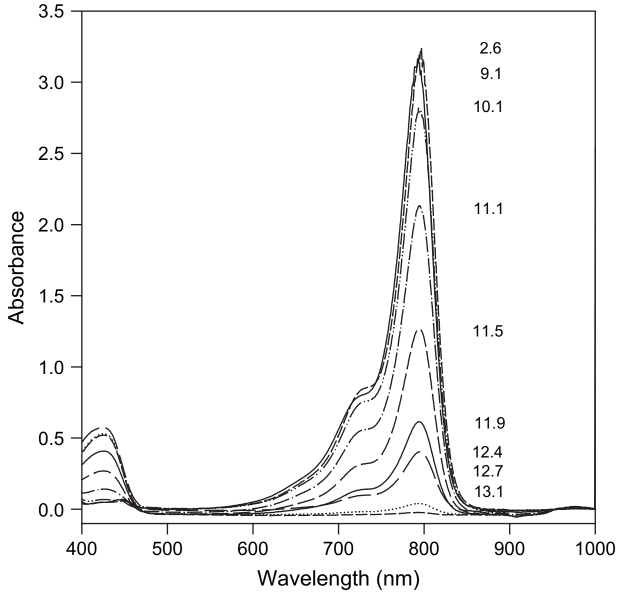


Fig. 4. Absorption spectra of dye **3b** 1.1×10^{-5} M in pure ethanol at different pH values.

4.3.1.3. Dye ***3c****.* Yield 240 mg (35%). λ_{max} (MeOH) 808.5 nm ($\varepsilon = 2.28 \times 10^5$ l/mol cm). FABMS 543 (100%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.29 (t, $J =$ 7.3 Hz, 6H), 1.63 (s, 12H), 1.84 (quint., $J = 5.3$ Hz, 2H), 2.68 (t, $J = 5.2$ Hz, 4H), 4.19 (q, $J = 7.4$ Hz, 4H), 6.20 (d, $J = 14.0$ Hz, 2H), 6.82 (dd, $J = 8.8$, $J = 2.2$ Hz, 2H), 7.01 (s, 2H), 7.26 (d, $J = 8.8$ Hz, 2H), 8.16 (d, $J = 14.0$ Hz, 2H), 9.74 (s, 2H).

4.3.1.4. Dye ***3d****.* Yield 320 mg (42%). λ_{max} (MeOH) 787.0 nm ($\varepsilon = 2.68 \times 10^5$ l/mol cm). FABMS 631 (100%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.30 (t, $J =$ 7.0 Hz, 6H), 1.79 (s, 12H), 1.86 (quint., $J = 4.9$ Hz, 2H), 2.75 (br. t, 4H), 4.25 (br. q, 4H), 6.39 (d, $J = 14.6$ Hz, 2H), 7.08 (d, 8.0 Hz, 2H), 7.93 (d, $J = 8.0$ Hz, 2H), 8.33 (d, $J = 14.6$ Hz, 2H).

4.3.1.5. Dye ***3e****.* Yield 354 mg (47%). λ_{max} (MeOH) 807.5 nm ($\varepsilon = 2.63 \times 10^5$ l/mol cm). FABMS 625 (100%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.30 (t, $J =$ 6.6 Hz, 6H), 1.64 (s, 12H), 1.85 (br. quint., 2H), 2.06 (s, 6H), 2.70 (br. t, 4H), 4.22 (q, $J = 6.6$ Hz, 4H), 6.27 (d, $J = 14.0$ Hz, 2H), 7.39 (d, $J = 8.8$ Hz, 2H), 7.53 (dd, $J = 8.8$ Hz, $J = 1.5$ Hz, 2H), 7.84 (d, $J = 1.5$ Hz, 2H), 8.20 (d, $J = 14.0$ Hz, 2H), 10.14 (s, 2H).

4.3.1.6. Dye ***3g****.* Yield 405 mg (65%). λ_{max} (MeOH) 794.5 nm ($\varepsilon = 1.95 \times 10^5$ l/mol cm). FABMS 491.1

(100%). NMR cannot be obtained due to the low solubility of the dye in the commonly used deuterated solvents.

4.3.1.7. Dye ***3h***. Yield 1.25 mg (94%). λ_{max} (MeOH) 794.0 nm ($\varepsilon = 2.10 \times 10^5$ l/mol cm). FABMS 707.0 (100%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.64–1.93 (m, 10H), 2.68 (br. t, 4H), 3.41 (overl. H_2O, 4H), 4.43 (br. t, 4H), 6.54 (d, $J = 13.9$ Hz, 2H), 7.31–7.37 (m, 2H), 7.48–7.55 (m, 2H), 7.75–7.80 (m, 4H), 7.94 (d, $J = 6.6$ Hz, 2H).

4.3.1.8. Dye ***3f***. It is obtained from the isomeric mixture of 4- and 6-hydroxysubstituted 1-ethyl-2,3,3-trimethylindoleninium iodides.

Recrystallisation of the crude product of cyanine condensation from methanol yields 410 mg (61%). λ_{max} (MeOH) 789.0 nm ($\varepsilon = 2.76 \times 10^5$ l/mol cm). FABMS 543 (100%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.29 (t, $J = 7.3$ Hz, 6H), 1.61 (s, 12H), 1.85 (quint., $J = 5.1$ Hz, 2H), 2.71 (t, $J = 5.1$ Hz, 4H), 4.17 (q, $J = 7.3$ Hz, 4H), 6.29 (d, $J = 13.8$ Hz, 2H), 6.68 (dd, $J = 8.0$ Hz, $J = 1.7$ Hz, 2H), 6.79 (d, $J = 1.5$ Hz, 2H), 7.38 (d, $J = 8.0$ Hz, 2H), 8.22 (d, $J = 13.8$ Hz, 2H), 9.80 (s, 2H).

4.4. Synthesis of dye ***3i***

Two hundred and fifty milligrams (0.33 mmol) of dye **3e** are dissolved in 6 ml of methanol, containing 150 mg of BF_3 and the mixture is refluxed for 2 h. After cooling, 0.5 ml of 25% aqueous NH_3 is added. The solvent is removed under vacuum and the residue is dissolved in a small amount of methanol and precipitated by addition of ether. Yield 634 mg (95%). λ_{max} (MeOH) 835.0 nm ($\varepsilon = 1.00 \times 10^5$ l/mol cm). FABMS 541 (100%). ^{1}H NMR (DMSO-d_6) 1.27 (t, $J = 7.3$ Hz, 6H), 1.60 (s, 12H), 1.83 (br. quint., 2H), 2.56 (br. t, 4H), 4.13 (br. q, 4H), 5.40 (br. s, 2H), 6.13 (d, $J = 13.9$ Hz, 2H), 6.60 (d, $J = 8.0$ Hz, 2H), 6.74 (d, $J = 2.2$ Hz, 2H), 7.13 (d, $J = 8.0$ Hz, 2H), 8.08 (d, $J = 13.9$ Hz, 2H).

4.5. Synthesis of dyes ***4***

4.5.1. Dye ***4a***

Yield 15 mg (47%). λ_{max} (MeOH) 667.5 nm ($\varepsilon = 6.54 \times 10^5$ l/mol cm). FABMS 560.3 (100%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.23 (t, $J = 6.6$ Hz, 6H), 1.60 (s, 12H), 1.73–1.83 (m, 8H), 2.50 (overl. DMSO, 4H), 3.76 (br. t, 4H), 4.04 (q, $J = 6.6$ Hz, 4H), 5.86 (d, $J = 13.2$ Hz, 2H), 7.07–7.68 (m, 10H).

4.5.2. Dye ***4b***

Yield 112 mg (46%). λ_{max} (MeOH) 777.5 nm ($\varepsilon = 3.11 \times 10^5$ l/mol cm). FABMS 600.3 (100%), 602.4 (15%) ^{1}H NMR (DMSO-d_6) δ (ppm) 1.29 (t, $J = 6.6$ Hz, 6H), 1.54 (s, 12H), 1.88 (quint., $J = 6.0$ Hz, 2H), 2.71 (br. t, 4H), 4.21 (q, $J = 6.6$ Hz, 4H), 5.20 (br. s, 2H), 6.30 (d, $J = 14.0$ Hz, 2H), 6.51(d, $J = 8.8$ Hz, 2H), 6.98 (d, $J = 8.8$ Hz, 2H), 7.22–7.28 (m, 2H), 7.43 (br. d, 4H), 7.56 (d, $J = 7.3$ Hz, 2H), 8.73 (d, $J = 14.0$ Hz, 2H).

4.5.3. Dye ***4c***

Yield 114 mg (42%). λ_{max} (MeOH) 802.5 nm ($\varepsilon = 1.46 \times 10^5$ l/mol cm). FABMS 688 (M^+, 100%), 671 (M^+–OH, 65%). ^{1}H NMR (CD_3OD) δ (ppm) 1.29 (t, $J = 6.4$ Hz, 6H), 1.49 (s, 12H), 1.85 (br. quint., 2H), 2.65 (br. t, 4H), 4.10 (q, $J = 6.4$ Hz, 4H), 6.22 (d, $J = 14.0$ Hz, 2H), 6.52 (d, $J = 8.6$ Hz, 2H), 6.92 (d, $J = 8.6$ Hz, 2H), 7.20 (d, $J = 8.1$ Hz, 2H), 7.88–8.00 (m, 4H), 8.88 (d, $J = 14.0$ Hz, 2H)

4.5.4. Dye ***4d***

Yield 105 mg (45%). λ_{max} (MeOH) 802.0 nm ($\varepsilon = 2.95 \times 10^5$ l/mol cm). FABMS 580.1 (M^+, 40%). ^{1}H NMR (DMSO-d_6) δ (ppm) 1.38 (br. t, 6H), 1.80 (br. quint., 2H), 2.62 (t, $J = 5.1$ Hz, 4H), 4.43 (q, $J = 7.2$ Hz, 4H), 5.30 (br. s, 1H), 6.43–6.53 (m, 4H), 7.10 (d, $J = 8.6$ Hz, 2H), 7.35–7.41 (m, 2H), 7.52–7.58 (m, 2H), 7.56 (br. d, 2H), 7.95 (d, 2H), 8.45 (d, $J = 14.0$ Hz, 2H).

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References

[1] Morf WE, Seiler K, Lehmann B, Behringer C, Tan SSS, Hartman K, et al. In: Pungor E, editor. Ion selective electrodes, vol. V. Akadémiai Kiadó; 1989.

[2] Morf WE, Seiler K, Sørensen PR, Simon W. In: Pungor E, editor. Ion selective electrodes, vol. V. Akadémiai Kiadó; 1989.

[3] Imasaka T, Ishibashi N. Diode lasers and practical trace analysis. Analytical Chemistry 1990;62(6):363A–71A.

[4] Patonay G, Antoine MD. Near-infrared fluorogenic labels: new approach to an old problem. Analytical Chemistry 1991;63(6):321A–7A.

[5] Hamer FM. The cyanine dyes and related compounds. John Wiley and Sons, Interscience Publishers; 1964.

[6] Boyer AE, Devanathan S, Hamilton D, Patonay G. Spectroscopic studies of a near-infrared absorbing pH sensitive carbocyanine dye. Talanta 1992;39(5):505–10.

[7] Lipowska M, Patonay G, Strekowski L. New near-infrared cyanine dyes for labelling of proteins. Synthetic Communications 1993;23(21):3087–94.

[8] Makin SM, Boiko II, Shavrygina OA. Synthesis and study of tricarbocyanines containing five- and six-membered rings in chromophore. Zhurnal Organicheskoi Khimii 1977;13(11): 1189–92.

[9] Strekowski L, Lipowska M, Patonay G. Facile derivatizations of heptamethine cyanine dyes. Synthetic Communications 1992;22(17):2593–8.

[10] Strekowski L, Lipowska M, Patonay G. Substitution reactions of a nucleofugal group in heptamethine cyanine dyes. Synthesis of an isothiocyanato derivative for labelling of proteins with a near-infrared chromophore. Journal of Organic Chemistry 1992;57(17):4578–80.

[11] Fry DJ. Heterocyclic compounds. In: Rood's chemistry of carbon compounds, IVB. Amsterdam: Elsevier Scientific; 1977 [chapter 15].

[12] Sturmer DM. In: Kirk-Othmer encyclopedia of chemical technology, 3rd ed., vol. VII. Wiley-Interscience; 1979.

[13] Lipowska M, Patterson SE, Patonay G, Strekowski L. A highly selective hydrogen–deuterium exchange in indolium heptamethine cyanines. Journal of Heterocyclic Chemistry 1993; 30(5):1177–80.

[14] Bakker E, Lerchi M, Rosatzin T, Rusterholz B, Simon W. Synthesis and characterization of neutral hydrogen ion-selective chromoionophores for use in bulk optodes. Analytica Chimica Acta 1993;278(2):211–25.

[15] West W, Pearce S. The dimeric state of cyanine dyes. The Journal of Physical Chemistry 1965;69(16):1894–903.

[16] Feldman LH, Herz AH, Regan TH. pH dependence of electronic and nuclear magnetic resonance spectra of isomeric cyanine dyes. The Journal of Physical Chemistry 1968;72(6):2008–13.

[17] Zen J-M, Patonay G. Near-infrared fluorescence probe for pH determination. Analytical Chemistry 1991;63(24):2934–8.

[18] Terpetschnig E, Szmacinski H, Ozinskas A, Lakowicz JR. Synthesis of squarine-*N*-hydroxysuccinimide esters and their biological application as long-wavelength fluorescent labels. Analytical Biochemistry 1994;217(2):197–204.

[19] Mujumdar RB, Ernst LA, Mujumdar SR, Waggoner AS. Cyanine dye labelling reagents containing isothiocyanate groups. Cytometry: Journal of the Society for Analytical Cytology 1989;10(1):11–9.