Effects of chemical structure, solvent and solution pH on the visible spectra of some new methine cyanine dyes

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1. Introduction

Cyanine dyes [1-10] have a very wide range of application, especially in the so-called “high-tech” fields, science and modern technology. They are used as luminescent covalent or non-covalent probes in biological, medicinal and clinical research and assay. Well known is their utilization in optical information storage for fabrication of CD-R and DVD-R (optical recording disks), in preparation of security marks over banknotes and documents, as novel materials in non-linear optics, as highly sensitive analytical reagents, as analytical sensors, in photodynamic therapy of cancer, for single molecule fluorescence detection, as photosensitizers in photographic processes [11-13] and as photo polymerization initiators [14].

The most numerous and continuously expanding applications of cyanine dyes are in the field of biotechnology. Among those is the visualization of DNA by following different biological processes and quantification of DNA in solution, YOYO-1 (intercalating dye) labeled complexes may be employed as qualitative DNA markers in intracellular delivery studies [15].

Taking in accounts and consideration the above vital and important benefits of cyanine dyes, the aim of this research paper is synthesis of some novel photosensitizers, solvato-chromic and halochromic cyanine dyes as new synthesis contribution and spectroscopic investigation in this field and/or to be used in any of the high-tech fields of cyanine dyes, particularly as photographic sensitizers, acid-base indicators and/or as probes for determining solvent polarity. In addition, because cyanine dyes have a multidisciplinary uses and applications in several fields and different areas of science, technology, engineering, pharmacology and medicine, this research paper may be very interesting to read by the many of the large heterogenous community groups of chemists, biologists, physicists, biotechnologists, pharmacologists and medical scientists.

2. Experimental

2.1. General

Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Elemental analyses were carried out at the Microanalytical Center at Cairo University. Infrared spectra were measured with a 1650 FT-IR instrument, and the 1H NMR spectra on an EM-390 90 MHz NMR Spectrometer, and mass spectroscopy was recorded on GCMS-QP-1000 EX Spectrometer (Cairo University). The organic solvents were of spectroscopic grades and the new dyes were investigated and purified using TLC chromatographic procedures.

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Table 1. Characterization of the prepared compounds.

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Nature of products</th>
<th>Molecular Formula (M.Wt., g)</th>
<th>Analysis % Calculated (Found)</th>
<th>Visible absorption spectra in 95% ethanol solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Color</td>
<td>Yield, %</td>
<td>M.p., °C</td>
<td>C</td>
</tr>
<tr>
<td>2a</td>
<td>Dark brown crystals</td>
<td>63</td>
<td>191-193</td>
<td>C₂₆H₁₄N₆O₆</td>
</tr>
<tr>
<td>2b</td>
<td>Dark brown crystals</td>
<td>43</td>
<td>186-188</td>
<td>C₂₆H₁₄N₆O₆ ₂₂₄₆.60</td>
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<tr>
<td>2c</td>
<td>Pale brown crystals</td>
<td>59</td>
<td>199-201</td>
<td>C₂₆H₁₄N₆O₆</td>
</tr>
<tr>
<td>3a</td>
<td>Reddish brown crystals</td>
<td>41</td>
<td>202-204</td>
<td>C₂₆H₁₄N₆O₆ ₅₀₆₄.4₃</td>
</tr>
<tr>
<td>3b</td>
<td>Brown crystals</td>
<td>38</td>
<td>187-189</td>
<td>C₂₆H₁₄N₆O₆ ₅₃₈₅.₅₅</td>
</tr>
<tr>
<td>3c</td>
<td>Intense brown crystals</td>
<td>47</td>
<td>213-215</td>
<td>C₂₆H₁₄N₆O₆ ₅₈₀₄.₄₆</td>
</tr>
<tr>
<td>4a</td>
<td>Reddish brown crystals</td>
<td>71</td>
<td>219-221</td>
<td>C₂₆H₁₄N₆O₆ ₇₂₁₉.₀₉</td>
</tr>
<tr>
<td>4b</td>
<td>Reddish violet crystals</td>
<td>65</td>
<td>227-229</td>
<td>C₂₆H₁₄N₆O₆ ₇₇₃₁.₁₂</td>
</tr>
<tr>
<td>4c</td>
<td>Reddish brown crystals</td>
<td>61</td>
<td>221-223</td>
<td>C₂₆H₁₄N₆O₆ ₇₂₁₉.₀₉</td>
</tr>
<tr>
<td>4d</td>
<td>Pale violet crystals</td>
<td>36</td>
<td>209-211</td>
<td>C₂₆H₁₄N₆O₆ ₇₇₃₁.₁₂</td>
</tr>
<tr>
<td>5a</td>
<td>Dark brown crystals</td>
<td>52</td>
<td>217-219</td>
<td>C₂₆H₁₄N₆O₆ ₇₇₃₁.₀₇</td>
</tr>
<tr>
<td>5b</td>
<td>Reddish violet crystals</td>
<td>52</td>
<td>217-219</td>
<td>C₂₆H₁₄N₆O₆ ₇₇₃₁.₀₇</td>
</tr>
<tr>
<td>5c</td>
<td>Dark brown crystals</td>
<td>44</td>
<td>218-220</td>
<td>C₂₆H₁₄N₆O₆ ₇₃₅₁.₀₇</td>
</tr>
<tr>
<td>5d</td>
<td>Violet crystals</td>
<td>49</td>
<td>211-213</td>
<td>C₂₆H₁₄N₆O₆ ₇₉₆₈.₀₆</td>
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<tr>
<td>5e</td>
<td>Dark violet crystals</td>
<td>39</td>
<td>228-230</td>
<td>C₂₆H₁₄N₆O₆ ₇₈₅₃.₃₁</td>
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<tr>
<td>5f</td>
<td>Reddish violet crystals</td>
<td>60</td>
<td>217-219</td>
<td>C₂₆H₁₄N₆O₆ ₉₆₈₈.₈₈</td>
</tr>
<tr>
<td>6a</td>
<td>Intense violet crystals</td>
<td>61</td>
<td>234-236</td>
<td>C₂₆H₁₄N₆O₆ ₁₀₇₉.₀₁</td>
</tr>
<tr>
<td>6b</td>
<td>Violet crystals</td>
<td>63</td>
<td>215-217</td>
<td>C₂₆H₁₄N₆O₆ ₁₀₇₉.₀₁</td>
</tr>
<tr>
<td>6c</td>
<td>Intense violet crystals</td>
<td>59</td>
<td>224-226</td>
<td>C₂₆H₁₄N₆O₆ ₁₁₀₄.₀₄</td>
</tr>
<tr>
<td>6d</td>
<td>Intense violet crystals</td>
<td>53</td>
<td>237-239</td>
<td>C₂₆H₁₄N₆O₆ ₁₁₀₄.₀₄</td>
</tr>
<tr>
<td>6e</td>
<td>Intense violet crystals</td>
<td>53</td>
<td>237-239</td>
<td>C₂₆H₁₄N₆O₆ ₁₁₀₄.₀₄</td>
</tr>
</tbody>
</table>

2.2. Synthesis

2.2.1. Synthesis of 4-formyl-10-methyl-2,8-diphenyl-5-ll-dihydrobenzo[2,3-b; 2',3'-b'] bis-pyrazolo[4,5-b]l4-(oxa-, thia- and pyra)-zine-6,12-dione (2a-c)

A pure crystallized sample of compounds 1a-c [16] was heated under reflux in equimolar ratios (0.01 mol) with selenium dioxide in dioxane (50 mL) for 16 hours. The reaction mixtures were filtered on hot to remove selenium metal and the filtrate was cooled, and then poured in ice water mixture. The precipitated products were filtered, dried, collected, and recrystallized from ethanol. The data are shown in Table 1.

2.2.2. Synthesis of 4,10-diformyl-2,8-diphenyl-5,ll-dihydrobenzo[2,3-b; 2',3'-b'] bis-pyrazolo[4,5-b]l4-(oxa-, thia- and pyra)-zine-6,12-dione (3a-c)

Two different routes are employed to prepare these compounds.

Route 1: This route is carried out in a way that has been described earlier [17].

Route 2: An equimolar ratios (0.01 mol) of both the 4-monoformyl derivatives (2a-c) and selenium dioxide were heated under reflux in dioxane (50 mL) for 16 hours. The reaction mixtures were filtered while hot to remove selenium metal, cooled, and poured in ice-water mixture. The precipitated products were filtered, dried, and recrystallized from ethanol to give the same diformyl compounds 3a-c obtained by Route 1 characterized by melting points, mixed melting points, same IR and ¹H NMR spectral data, Table 1 and 2.

2.2.3. Synthesis of 10-methyl-2,8-diphenyl-5,ll-dihydrobenzo[2,3-b; 2',3'-b'] bis-pyrazolo[4,5-b]l4-(oxa-, thia- and pyra)-zine-6,12-dione-4f(4j) dimethine cyanine dyes (4a-e)

Reaction mixtures of equimolar ratios (0.01) of compounds 2a-c and ethyl iodide quaternary salts of 2-picolinum, quinaldine, 4-picolinum were refluxed in ethanol (30 mL) containing few drops (3-5) of piperidine for 8-10 hours. During the refluxed time the colors changed from deep brown to intense violet at the end of the refluxing. The reaction mixtures were filtered while hot to remove unreacted materials, concentrated by evaporation, cooled in ice bath, neutralized with glacial acetic acid and precipitated by adding cold water. The precipitates were collected, dried, and recrystallized from ethanol. The relevant data are given in Table 1 and 2.

2.2.4. Synthesis of 10-formyl-2,8-diphenyl-5,ll-dihydrobenzo[2,3-b; 2',3'-b'] bis-pyrazolo[4,5-b]l4-(oxa-, thia- and pyra)-zine-6,12-dione-4f(4j) dimethine cyanine dyes (5a-e)

N-Ethyl-(2-picolinium, quinaldinium, 4-picolinium) iodide quaternary salts were heated under reflux in equimolar ratios (0.01 mol) with compounds 3a-c in ethanol (30 mL) containing piperidine (3-5 drops) for 8 hours.
2. Results and discussion

3.1. Synthesis

Selenium dioxide oxidation of 4,10-dimethyl-2,8-diphenyl-5,11-dihydrobenzo[2,3-b;2',3'-b']bis-pyrazolo-[4,5-b],[4,5-b],4,4(oxa, thia-, and pyra-)azine-6,12-dione (1a-c) in 1:1 molar ratios resulted in the same compounds, Scheme 1, Table 1. Other equimolar selenium dioxide oxidation of the mono formyl compounds 2a-c in dioxane as organic solvent gave the dimethine dyes (4b-e). Reaction of the 4-formyl compounds 2a-c and 1-ethyl (2-picolinium, quinaldinium, 4-picolinium) iodide quaternary salts in ethanol containing piperidine gave 4(2[4]) mono cationic dimethine dye 4a-e. Also interaction of the 4,10-diformyl compounds 3a-c with equimolar and/or bimolar ratios of the iodoethane quaternary salt of 2-picoline, quinaldine and/or 4-picoline in ethanol containing piperidine yielded 4(2[4]) dimethine cyanine dyes (5a-e) and/or (6a-e), respectively, Scheme 1, Table 1. Chemical confirmation takes place via route 2, through reaction of the 4(2[4]) dimethine cyanine dyes (5a-e) and equimolar ratios of N-ethyl-(2-picolinium, quinaldinium, and/or 4-picolinium) iodide quaternary salts in ethanol catalyzed by piperidine to achieve the same, 4,10(2[4]) bis-dimethine cyanine dyes (6a-e), characterized by same melting points, mixed melting points, same IR and 1H NMR spectral data, Scheme 1, Table 1 and 2.

The structures of the prepared compounds were identified by elemental analysis, Table 1, mass spectroscopy, IR [19] and 1H NMR [20] spectra, Table 2.

3.2. Effect of chemical structure on the visible spectra

Effect of chemical structure on the visible spectra of all the prepared cyanine dyes were investigated by measuring their electronic visible absorption spectra in 95% ethanol solution, Table 1.

The electronic visible absorption spectra of the dimethine cyanine dyes (4a-e), (5a-e), and the bis-dimethine cyanine dyes (6a-e) in 95% ethanol solution showed absorption spectra bands in the visible regions, (393.0-561.0 nm), (381.0-557.5 nm), and (411.0-599.5 nm), respectively. These bands undergo to give bathochromic and/or hypsochromic shifts depending largely on the nature of the heterocyclic quaternary salts, their linkage position and the type of the benz-bis-heterocyclic system present, Table 1. Thus, the absorption spectrum of the dimethine cyanine dyes 4a, 5a, and 6a (X = 0, A = 1-ethyl pyridinium-2-yl salt) showed bands at λ 5285, 524.5, and 543.0 nm, respectively.
Substituting A = 1-ethyl quinolinium-2-yl salt to give dyes 4b, 5b, and 6b causes a bathochromic shifts of 23.5, 10.5, and 16.5 nm with intensification of the absorption bands in addition to the appearance of new absorption bands at (393.0, 445.0 nm), (381.0, 434.5 nm) and (411.0, 491.0 nm) for the latter dyes, respectively. This is related to extra conjugation in the quinoline moiety in the former dyes with the pyridine moiety in the former dyes, Scheme 1, Table 1. Also, changing the linkage position in the pyridinium residue from 2-yl salts in dyes 4a, 5a, and 6a to 4-yl salts in dyes 4c, 5c, and 6c, resulted in a bathochromic shift of 5, 4, and 8 nm, respectively, accompanied by increasing the intensities of the absorption bands. This is due to increasing the π-delocalization conjugation in the latter 4-yl salt dyes compared with the former 2-yl salts, Scheme 1, Table 1. Additionally, the dimethine and bis-dimethine cyanine dyes 4b, 5b, and 6b incorporating pyrazolo-oxazine nucleus showed bathochromically shifted bands by (4 and 9 nm); (16.0 and 22.5 nm), and (11.5 and 30.0 nm), respectively, compared by its analogous dyes, 4d, 5d, 4e; 5d, 5e; and 6d, 6e which contain pyrazolo-thiazine and pyrazolo-pyrazine nuclei, respectively. This is due to increasing the electron attracting character of the oxazine ring in dyes 4b, 5b, and 6b compared with thiazine and pyrazine rings in dyes 4d, 4e, 5d, 5e, and 6d, 6e, respectively. This leads to decreasing the electronic charge transfer pathways to the heterocyclic quaternary nitrogen atom of quinolinium salt residue, and consequently a hypsochromic shift is resulted in oxazine dyes. On the other side it was observed that substituting the 10-methyl group in dyes (4a-e) by 10-formyl group to give dyes (5a-e), made hypsochromic shifts in addition to decreasing the intensities of the absorption spectra bands. This could be attributed to the electron donating character of methyl group in dyes (4a-e) and electron attracting character of the formyl group in dyes (5a-e). Scheme 1, Table 1. Moreover, it is noticed that the number of electronic charge transfer pathways have a remarkable effect on the spectral behavior of these dyes, so, the bis-dimethine cyanine dyes (6a-e) showed absorption spectra bands which are bathochromically shifted if compared with its analogous dimethine cyanine dyes (5a-e) and/or (4a-e) with intensification of the absorption spectra bands, Table 1. This is can be explained by the presence of two electronic charge transfer pathways in the bis-dimethine cyanine dyes (6a-e) in correspondence to one electronic charge transfer pathways in the dimethine cyanine dyes (5a-e) and/or (4a-e), Scheme 2 (A), [B], Table 1.

3.3. Effect of solvent on the visible spectra

Effect of solvent in the visible spectra of the prepared bis-dimethine cyanine dye 6b was investigated by measuring its electronic visible absorption spectra in pure solvents having different polarities, Table 3.

The electronic visible absorption spectra of the bis-dimethine cyanine dye 6b in pure solvents of different dielectric constant via water (78.54), DMF (36.70), ethanol (24.3), chloroform (4.806), carbontetrachloride (2.238) and dioxane (2.209) [21] are recorded. The λ (wavelengths) and ε (molar extinction coefficients) values of the absorption bands due to different electronic transitions within the solute molecule in these solvents are represented in Table 3.

From Table 3, its clearly that, the electronic visible absorption spectra of the dye 6b in ethanolic medium are characterized by the presence of three essential absorption bands. These bands can be assigned to intramolecular charge transfer transitions [22]. These charge transfer is due to transfer of lone pair of electrons from the N-Ph pyrazole nitrogen atom to the positively charged quaternary nitrogen atom of the quinolinium salt, Scheme 2 (B).

The data given in Table 3 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 increasing of dielectric constant of DMF relative to ethanol. (b) The hypsochromic shift occur in ethanol relative to dioxane, chloroform and carbontetrachloride is a result of the solute solvent interaction through inter-molecular hydrogen bond formation between ethanol and the lone pair electrons of N-Ph pyrazole nitrogen atom, Scheme 3 (A). This decreases slightly 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 quaternary nitrogen atom of the quinolinium quaternary salt.
Also, from the data given in Table 3, it is observed that occurrence of unexpected hypsochromic shifts in water relative to ethanol and the other solvents as well as decreasing the number and intensity of the absorption bands. 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 transfer of electronic charge from the N-Ph pyrazole nitrogen atom to the quaternary heterocyclic residue of the quinolinium salt, and consequently there is observed a hypsochromic shift in water relative to ethanol and the other solvents.

### 3.4. Effect of solution pH on the visible spectra

Effect of solution pH on the visible spectra of the synthesized bi-dimethine cyanine dye 6b was investigated by measuring its spectra in aqueous universal buffer solutions.

The solutions of the bis-dimethine cyanine dye 6b have a permanent cationic charge in basic media that then discharged on acidification. This prompted and encouraged us to study its spectral behavior in different buffer solutions in order to select a suitable pH for use of this dye as photosensitizers. The acid dissociation or protonation constant of this dye has been determined. The effect of the compound as photosensitizers increases when they are present in the ionic form, which has a higher planarity [23], and therefore more conjugation.

The electronic absorption spectra of the bis-dimethine cyanine dye 6b 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 hypsochromic shifts with quenching the intensity of the bands at low pH media (acidic media). So, the mentioned dye which has lone pair of electrons 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 quaternary heterocyclic quinolinium salt will be difficult resulting 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 quaternary heterocyclic quinolinium salt will be easier and facilitated, Scheme 3 (B).

Several methods have 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 fixed λ vs. pH, S-shaped curves are obtained, Table 4. An 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 pka 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 the left and right segments [24]. The acid dissociation or protonation constants values of the dye 6b are given in Table 4.

### Table 3. Electronic visible absorption spectra of the bis-dimethine cyanine dye (6b) in pure solvents.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>H2O</th>
<th>EtOH</th>
<th>DMF</th>
<th>CHCl3</th>
<th>CCl4</th>
<th>Dioxane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dye no</td>
<td>λ*</td>
<td>ε**</td>
<td>λ*</td>
<td>ε**</td>
<td>λ*</td>
<td>ε**</td>
</tr>
<tr>
<td>(6b)</td>
<td>484</td>
<td>20220</td>
<td>411</td>
<td>27410</td>
<td>503</td>
<td>26860</td>
</tr>
<tr>
<td></td>
<td>551</td>
<td>19220</td>
<td>491</td>
<td>26130</td>
<td>579</td>
<td>28830</td>
</tr>
</tbody>
</table>

* Unit: nm.
** Unit: mol⁻¹ cm².

![Scheme 2](image-url)
Table 4. The variation of absorbance (λ = 550 nm) with pH at settled λ for the cyanine dye (6b) in different buffer solution *.

<table>
<thead>
<tr>
<th>pH</th>
<th>1.87</th>
<th>2.51</th>
<th>3.92</th>
<th>5.06</th>
<th>6.89</th>
<th>8.13</th>
<th>9.56</th>
<th>11.41</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbance at settled wavelength</td>
<td>1.376</td>
<td>1.409</td>
<td>1.657</td>
<td>1.900</td>
<td>1.957</td>
<td>2.188</td>
<td>2.351</td>
<td>2.387</td>
</tr>
</tbody>
</table>

* pH = 4.380 and 8.235.

Scheme 3

4. Conclusion

From the results discussed in this study we can conclude that, these cyanine dyes can be used as (i) Photographic sensitizers for silver halide emulsions in photographic industry due to their spectral and/or photosensitization properties, (ii) Indicators for solvent polarity due to their solvatochromic properties, (iii) Acid-base indicators in analytical chemistry due to their halochromic properties.

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References