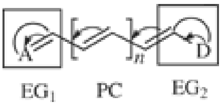
POLYMETHINE DYES

1. Introduction

Polymethine dyes (PD) represent a class of dyes, with their molecules normally containing the π-electron conjugated system (*polymethine chromophore*) that includes an *electron-acceptor end group* (EG_1), an *electron-donor end group* (EG_2), and the *polymethine chain* (PC) between them (general eq. 1) (1–5):



(1)

An electron-acceptor end group mostly contains an atom A = of high electronegativity adjacent to a double bond as, eg, N^+=, O^+=, S^+=, O=, and the carbon atom =C incorporated into a heterocyclic, normally monovalent residue. An electron-donor end group usually contains an atom D: bearing a lone pair of electrons, such as N:, O:, S:, and bound to the carbon atom in a heterocyclic divalent residue. The polymethine chain is constituted of sp^2-hybridized carbon atoms forming a chain of conjugated bonds. Conjugation between the electron-donor and the electron-acceptor groups results in the displacement of π electrons, and hence bond order equalization in the chromophore. Generally, the chromophore includes an odd number of atoms, viz., two end atoms, A and D, and an odd number of carbon atoms between them. Depending on the number of carbon atoms included in the end groups, the number of methine units between the end groups can be odd or even. The total number of π electrons in the chromophore is normally even and exceeds the number of atoms by 1.

If the end groups have the same chemical constitution, the PD is called symmetrical and its molecules belong to the C_{2v} symmetry group. The electron density distribution in the chromophore of such dyes is also symmetrical; in particular, the charges on the end heteroatoms and on the symmetrical carbon atoms are equal. If the end groups differ in chemical constitution, the polymethine dye is called unsymmetrical.

According to the chromophore type, the following basic types of the PD (R = Alk, Ar) are distinguished.

(a) Symmetrical dyes:

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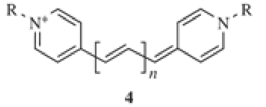
The simplest cyanine chromophores, streptocyanines

3

Oxonols



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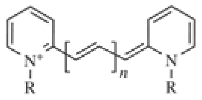
4-Pyridocyanines



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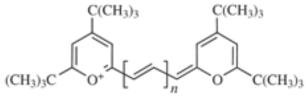
2-Pyridocyanines



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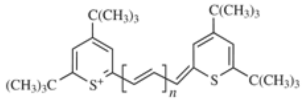
2-Pyrylocyanines



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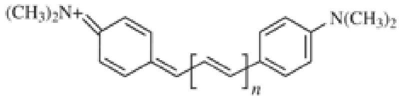
2-Thiopyrylocyanines



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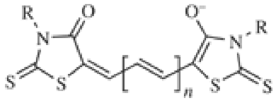
Diphenylpolymethine dyes



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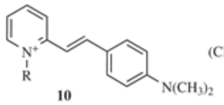
Rhodanine dyes

(b) Unsymmetrical dyes:



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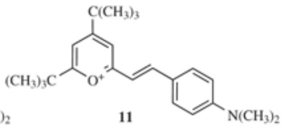

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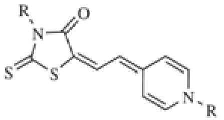
Styryls

12

The simplest merocyanine chromophores



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Merocyanine dye

In the molecule of a PD, one end group is in the electron-acceptor form and the other is in the electron-donor form. The latter contains one more electron. Either end group can be represented in both forms. Any substituents, including cyclic bridges, can be bound to the carbon atoms of the PC instead of the hydrogen atoms.

2. Absorption Spectra

The main feature of a PD is its ability to absorb light of a certain wavelength (characterized by the position of the absorption maximum, $\lambda_{\max}$) and with certain intensity (characterized by the extinction coefficient, ∂).

2.1. Vinylene Shifts of Absorption Maxima. In the above-mentioned molecules of polymethine dyes, the number of vinylene groups in the chromophore is $n = 0, 1, 2$, etc. As the value of n increases by 1, dye absorption maxima normally shift to longer wavelengths by ~100 nm, and is termed the *vinylene shift* (V, nm). Spectral characteristics of polymethine dyes **2** (2) are listed in Table 1.

By using the least-squares method, the following linear correlation between the absorption maximum wavelength and the number of vinylene groups can be found:

$$\lambda_{\max} = 310.8 + 105.3n \quad \text{Correlation coefficient } R = 0.999$$

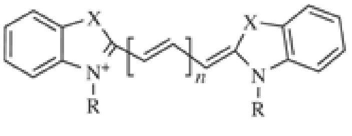
The coefficient of n in this correlation equation is equal to the average vinylene shift in the dye series concerned ($V = 105.3$ nm) and the number 310.8 is close to the absorption maximum of dye **2** with $n = 0$. We introduce the concept of the *spectral length of an end group* (L_0) in a symmetrical dye and express it as

$$L_0 = 0.5(\lambda_{\max} - 0.5V - Vn)$$

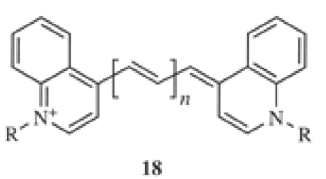
Then, the spectral length of the end group $(CH_3)_2N^+$ ∀ CH! [or ∀ CH! $N(CH_3)_2$] in dyes **2** is equal to 129.1 nm, and its absorption maximum wavelengths can be written as:

$$\lambda_{\max} = 2L_0 = 0.5V + Vn$$

To clarify, we present the correlation equations for the absorption maximum wavelengths as well as the average vinylene shifts and the end-group spectral lengths for the above-mentioned dye series and also for the most extensively studied polymethine dyes including those derived from indolenine (**14**), benzoxazole (**15**), benzothiazole (**16**), and quinoline (**17**,**18**) (Table 2).



X = $C(CH_3)_2$ (14), O (15), S (16), CH=CH (17)



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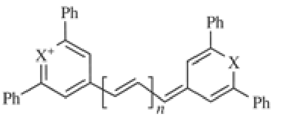
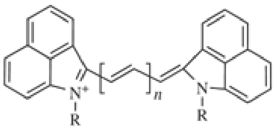
It is also informative to list, for comparison, the spectral data for extremely deeply colored dyes derived from 4-pyrylium (**19**), 4-thiopyrylium (**20**), and benz[*c*,*d*]indole (**21**) (3,4) (Table 2).



X = O (**19**), S (**20**)



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As seen from Table 2, the vinylene shifts range from 92 to 130 nm. The end groups in dye series **7**, **6**, **21**, and **20** stand out for their large spectral lengths. It is therefore these end groups that are incorporated into the dyes having relatively short polymethine chains and nevertheless absorbing light in the far infrared (ir) region, eg, dyes **6** ($n = 2$, $\lambda_{max} = 876$ nm), **21** ($n = 3$, $\lambda_{max} = 995$ nm), **20** ($n = 3$, $\lambda_{max} = 1020$ nm).

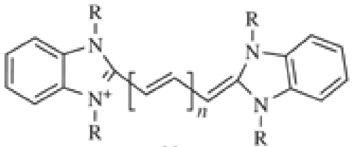
2.2. Unsymmetrical Dyes. The absorption maximum of an unsymmetrical dye is usually shifted to shorter wavelengths by the value $\Delta\lambda$ as against the arithmetical mean of the absorption maximum wavelengths for the corresponding symmetrical (parent) dyes. This quantity, referred to as a *deviation*, is measured in nanometers (nm) and specified by the formula

$$\Delta\lambda = 0.5(\lambda_{max,1} + \lambda_{max,2}) - \lambda_{max}$$

Deviations are mostly calculated and compared for trimethine unsymmetrical cyanine dyes ($n = 1$), styryl dyes (such as series **10**), and dimethine merocyanine dyes (such as series **13**). As an illustration, below is given a series of increasing deviations for styryl dyes with various end groups (labeled by the same number as the corresponding dye series) (2):

$0 < 31.5 < 50.2 < 59 < 77.5 < 107 < 109 < 114.5 < 125 < 140$ nm
$\mathbf{8} < \mathbf{14} < \mathbf{15} < \mathbf{16} < \mathbf{17} < \mathbf{18} < \mathbf{9} < \mathbf{5} < \mathbf{4} < \mathbf{22}$

A rise in deviations is indicative of increasing "electronic asymmetry" of the dye molecules, ie, increasingly different electron-donor abilities of two end groups, one of them, the dimethylaminophenyl residue, remaining the same for all styryls and the other being varied from dimethylaminophenyl as in series **8** (the lowest electron-donor ability) to benzimidazolyl as in series **22**, with $R = C_2H_5$ (the highest electron-donor ability).



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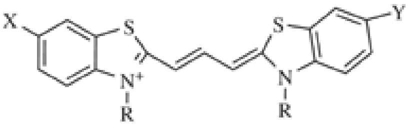
As a rule, absorption bands of polymethine dyes are narrow and intensive at a relatively small number of vinylene groups in the chain ($n = 1$–5). These bonds become weaker and wider or even completely diffuse at a large value of n. This regularity was observed, in particular, for dyes **16** and **4**. For example, dyes **4** in dimethyl sulfoxide (DMSO) manifest $\lambda_{max} = 486$, 606, 707 nm and lg $\partial = 5.15$, 5.20, 5.24 at $n = 0$, 1, 2, respectively, and $\lambda_{max} = 920$ nm, lg $\partial = 4.60$ at $n = 4$, whereas $\lambda_{max} = 738$, lg $\partial = 4.23$ at $n = 6$. The effect is caused by a limited size of the soliton wave of single electrons on molecular orbitals as well as by symmetry breaking for molecular orbitals that occurs at a sufficiently large length of the PC.

2.3. Effects of Substituents in Heterocyclic Nuclei. Substituents in the heterocyclic nuclei of symmetrical dyes usually give rise to the increased spectral lengths of the end groups and thus shift absorption maxima to longer wavelengths (so-called bathochromic effect), as illustrated by dyes **23**.

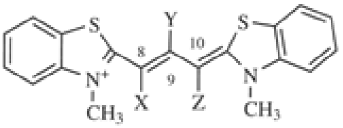
However, in the case where the substituents in two end nuclei are of opposite electronic nature, the end-group electron-donor abilities are changed in opposite directions, which results in deviation and a weakened bathochromic effect of such substituents.



23

X	Y	λ_{max}, nm
H	H	558
$N(CH_3)_2$	$N(CH_3)_2$	612
NO_2	NO_2	585
$N(CH_3)_2$	NO_2	588

2.4. Effects of Substituents in Polymethine Chain. The effects caused on dye absorption spectra by the substituents in the PC mostly obey the Förster-Dewar-Knott rule (FDK rule): electron-donor substituents at electron-rich positions of the chain (eg, at positions 8 and 10 of dyes **16**) give rise to bathochromic shifts, whereas at electron-deficient positions of the chain (eg, at position 9 of dyes **16**), they lead to hypsochromic shifts. The effects are accordingly reversed for electron-acceptor substituents at the corresponding positions. These regularities are most vividly demonstrated using dyes **24** (benzothiazole derivatives) as an example (6).

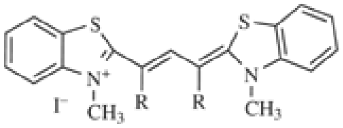


24

X	Y	Z	$\lambda_{max}(C_2H_5OH)$	$\Delta\lambda$
H	H	H	558	
H	NH_2	H	471	−87
H	F	H	522	−36
F	H	F	592	+44
H	CN	H	613	+55
CN	H	CN	490	−68
H	Ph	H	560	+2

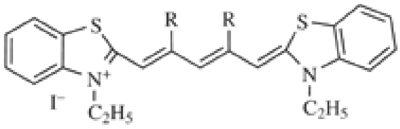
The absorption spectrum is mainly influenced by the conjugation effect. A fluorine atom in the chain therefore acts as an electron-donor substituent. A phenyl group at position 9 is twisted out of the chromophore plane, and hence exhibits no conjugation effect slightly influencing the absorption spectrum as an electron-acceptor substituent.

Alkyl groups and cyclic fragments incorporated into the chain affect the absorption maximum as electron-donor substituents in accordance with the FDK rule. Their action is exemplified by dyes **25, 26**, **27**; a dimethylene bridge is shown to exert a stronger effect than a trimethylene bridge (7,8).



25

R	R	λ_{max} (C_2H_5OH)	$\Delta\lambda$
H	H	558	
$-(CH_2)_2-$		594	+36
$-(CH_2)_3-$		565	+7
−CH=CH−		470	−88
$-1,2\text{-}C_6H_4-$		518	−40



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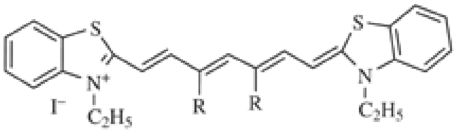
R	R	λ_{max} (C_2H_5OH)	$\Delta\lambda$
H	H	650	
$-(CH_2)_2-$		599	−51
$-(CH_2)_3-$		643	−7
$-1,2\text{-}C_6H_4-$		755	+105



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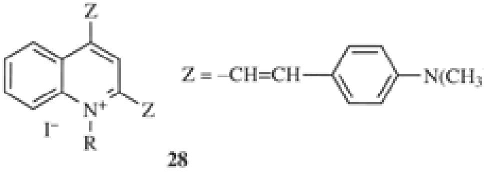
R	R	λ_{max} (CH_3OH)	$\Delta\lambda$
H	H	758	
$-(CH_2)_2-$		802	+44
$-(CH_2)_3-$		766	+8

A vinylene group in dye **25** displays a pronounced hypsochromic effect. An *o*-phenylene group acts likewise hypsochromically in trimethine dye **25**, but causes a strong bathochromic shift in pentamethine dye **26**.

2.5. Chromophore Interaction. If two chromophores are contained in a dye molecule, the absorption spectrum exhibits their interaction, which results in the bathochromic shift of the long-wavelength band and the hypsochromic shift of the short-wavelength band (9). Moreover, the relative absorption intensities can change: If the angle between the chromophores is >90°, the long-wavelength band becomes more intensive; at angles <90°, the relative intensity of the short-wavelength band rises. For example, dye **28** incorporates two chromophores characteristic of styryl dyes with the 2-quinolyl ($\lambda_{max} = 530$ nm) and the 4-quinolyl ($\lambda_{max} = 530$ nm) end groups. Accordingly, the absorption spectrum of this dye shows two bands at 500 and 570 nm that can be regarded as a result of chromophore interaction.



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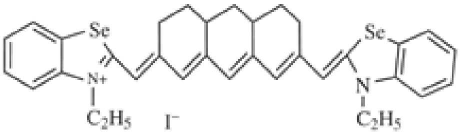
Sometimes chromophore interaction manifests itself in the cases when the chromophores are not bound chemically and are merely close in space, as in the aggregates of polymethine dye molecules.

3. Fluorescence Spectra

Spectral luminescent properties of polymethine dyes are characterized by the positions of fluorescence band maxima (λ_{fl}) and fluorescence quantum yields (φ). Polymethine dyes, like series **14–16**, normally exhibit the fluorescence maxima red-shifted by 18–23 nm relative to their absorption maxima (*Stokes'* shifts)[*]

(3,4,10). For dyes with the open PC, the quantum yields are low at $n = 0$ and $n > 3$ ($\varphi < 1\%$), but increase as the value of n grows from 0 to 3 ($\varphi = 25–58\%$ at $n = 2, 3$). In contrast to absorption bands that broaden and weaken at large values of n, fluorescence bands become narrower with rising n and their shape is practically independent of the solvent nature. If the PC is partially fixed by bridge groups, the quantum yields somewhat increase (7,8). To exemplify, dye **29** shows $\varphi = 0.95\%$, while its analogue with unsubstituted chromophore shows $\varphi = 0.17\%$.



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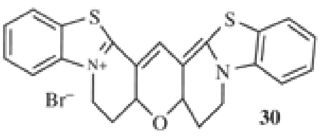
The quantum yield rises drastically if all the atoms in a dye molecule are fixed rigidly, as is the case in dyes **30** ($\varphi = 53\%$) and **31** ($\varphi = 97\%$) (3,4).



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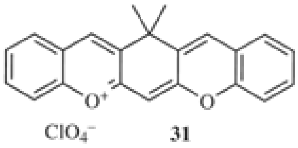

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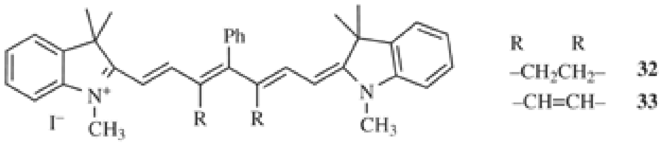
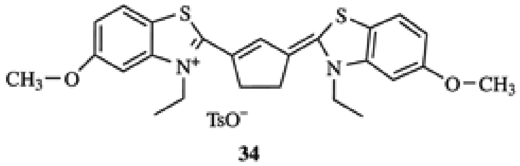
The value φ rises distinctly in passing from saturated bridge groups to a vinylene group. For example, the polymethine dyes **32** and **33** have fluorescence quantum yields of 8 and 55%, respectively in ethanol (3,4,8).



4. Application of Polymethine Dyes

4.1. Photographic Sensitization. If not doped with special additives (sensitizers), photographic silver halide emulsions applied in the production of light-sensitive materials (photofilms, cinefilms, photographic paper, etc) manifest sensitivity only to light of short wavelengths (up to 520 nm). Adding small amounts of sensitizers to these materials can make them sensitive to longer wavelengths (green and red light) and even to ir radiation (up to 1600 nm), thus affording dark photography. Among extensively used photosensitizers are polymethine dyes derived from benzothiazole, benzoxazole, benzimidazole, quinoline, etc (11).

Sensitization maxima are usually 20–80-nm red-shifted as compared to absorption maxima of dyes in solutions. As an example, the dyes of the benzothiazole series **16** with $n = 1–5$ display their sensitization maxima at 595, 695, 810, 915, and 1020 nm, respectively. In case of formation of long-wave units by PD on the AgHal microcrystales, the sensitization maximum is displaced more. For example, dye **34**, the absorption maximum of which is 615 nm, has the sensitization maximum 720 nm.



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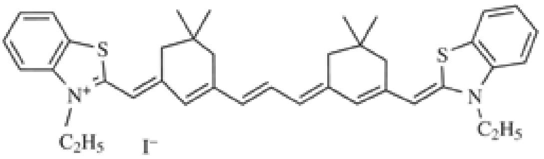
The applicability of ir-sensitizing PD depends on their stability during storage, their ability to absorb ir light, and their efficiency in spectral sensitization. Infrared PD generally have a low stability during storage and low spectral sensitization efficiency. Enhancement of the rigidity of the PC through alicyclic rings is very effective in improving the stability and sensitizing efficiency, hence applicability of ir sensitizing dyes, eg, 35.



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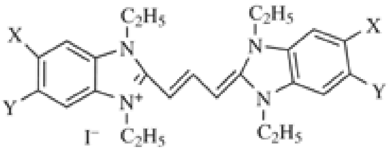
Sensitizing dyes added to photoemulsions should be highly purified and dosed thoroughly to provide the highest sensitization efficiency. The dye consumption is quite moderate: An amount of several milligrams is sufficient for 1 m^2 of an emulsion layer.

For high efficiency sensitization to be realized, it is significant to take into consideration not only the absorption maxima of polymethine dyes, but also their other properties, such as basicity, electron-donor ability of end groups, a degree of electronic symmetry, ability to form aggregates, etc. To exemplify, dyes **36** and **37** derived from benzimidazole, with their basicity lowered by the electron-acceptor substituents in heterocyclic nuclei, proved to be good sensitizers.

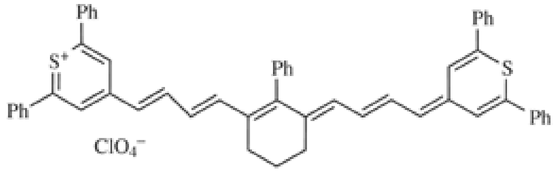


X = Y = Cl **36**

X = H, Y = $-SO_2CF_3$ **37**

Polymethine dyes have found multiple use in photography including optical sensitization, color imaging, production of colored light filters, etc (3,4).

4.2. Laser Technology. Since the mid-1970's, polymethine dyes have been widely applied in quantum electronics as mode-locking compounds in passive mode-locked lasers as well as active laser media. Using them for passive mode locking enabled generation of high power pulses with record short-time lengths, namely, femtosecond in the visible spectral region (with jet dye lasers) and picosecond in the near-IR (with solid-state lasers). The passive mode-locking regime for lasers emitting at ~1300 nm was first realized with dyes **38** and **39** ($n = 1$, $\lambda_{\max} = 1315$ nm, lg $\partial = 5.14$, CH_2Cl_2). Dye **39** [$n = 0$, $\lambda_{\mathrm{vax}} = 1055$ nm (C_2H_5OH)] is used in the passive mode-locking regime for neodymium lasers (1,3,4,12–14).



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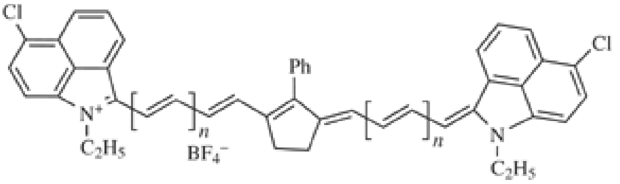

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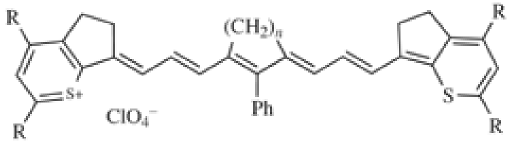
For erbium lasers emitting in the region of 1500−1700 nm, this became possible owing to dyes **40** ($\lambda_{\max} = 1470$ nm, lg $\partial = 5.01$, CH_2Cl_2) and **41** ($\lambda_{\max} = 1620$, lg $\partial = 4.97$, o-$C_6H_4Cl_2$). With the concentrated solutions of thiopyrylocyanine dye **42**, pumped by neodymium laser at 1056 nm, lasing wavelengths up 1800 nm were realized.



R = Ph, n = 2 **40**
R = Th, n = 2 **41**
R = Ph, n = 3 **42**

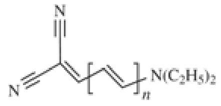
Nowadays, polymethine dyes represent the only class of organic compounds that afford, as active laser media, generation and wavelength tuning of laser emission for the wavelengths >1000 nm.

4.3. Nonlinear Optics. The application of polymethine dyes in nonlinear optics is based on the fact that they manifest nonlinear optical (NLO) properties in strong electric fields generated by laser emission (1,7,12−14). Polarization of organic materials in an electric field is described by the following equation:

$$P_{\mathrm{i}} = \alpha_{\mathrm{ij}}E_{\mathrm{ij}} + \beta_{\mathrm{ijk}}E_{\mathrm{j}}E_{\mathrm{k}} + \gamma_{\mathrm{ijkl}}E_{\mathrm{j}}E_{\mathrm{k}}E_{\mathrm{l}} + \cdots$$

where α is polarizability, and β and γ are first and second hyperpolarizabilities, respectively. NLO behavior is exhibited by the materials with the sizeable magnitudes of first and second hyperpolarizabilities and implies, in particular, second harmonic generation (SHG).

Important applications of NLO materials are frequency conversion, integrated optical devices, and frequency-optical modulation of laser radiation by NLO materials used, eg, in optical communication, optical image memory processing, and phase array control systems. For example, single-mode optical wave guides based on polymers with incorporated electrooptical dyes are conveniently employed to produce multichip modules for high-speed interconnects. Experimentally, first hyperpolarizability is evaluated by electric-field-induced second harmonic generation (EFISH) in solution using a strong direct current (dc) field for molecular alignment. Merocyanine dyes **12** ($n = 0$, 1, and 2) show an increase of second hyperpolarizabilities with elongation of the chain ($\beta \times 10^{30} = 3.3$, 20, and 53 esu, respectively). For dyes **43** with $n = 0$, 1, 2, and 3, $\beta \times 10^{30} = 1.0$, 6.1, 45, and 211 esu, respectively.

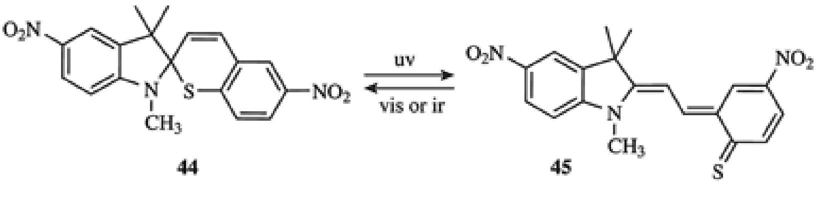


43

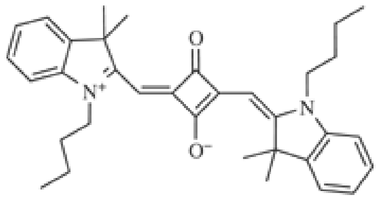
Since applications in nonlinear optics necessiate the use of polymer film materials, the electrooptic merocyanine dyes were often incorporated in polymers. The efficiency of SHG and the quality of the layer material is improved through covalent linkage of the cyanine and merocyanine dyes to polymers or to glass surfaces.

4.4. Optical Recording. Modern information recording and storage are based on electronic processes, especially on laser optical memory systems, such as compact disk records and videos (1,7,12–14). In optical compact disks, the light of the commercially available diode lasers emitting at 780 nm is used for the writing process and emission at 830−840 nm for the reproducing process. For use in such disks, organic dyes should have absorption bands in the near-ir region, as is the case with dyes **14** ($n = 3$, R = Alk, Ar).

In the reversible optical storage systems, photochromic dyes like **44** are used. The colorless spiropyran **44**, on a suitable substrate, undergoes color generation by converting to **45** ($\lambda_{max} = 750$ nm) on irradiation with ultraviolet (uv) light. Irradiation of **45** with a diode laser or with visible light gives **44**.



Many squarylium dyes, eg, dye **46** ($\lambda_{max} = 644$ nm in toluene), are offered as optical sensitizers in electrophotographic materials for record of phase holograms by the helium–neon laser radiating at 630–640 nm (15).



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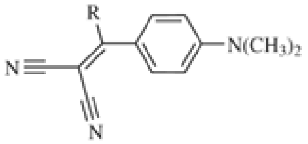
4.5. Electronic Photography.

In order to obtain color copies or prints from magnetic or video disks, the information stored must be converted into yellow, magenta, and cyan color dots by printers. Merocyanine-like dyes **47** and **48** are suitable for generation of yellow and magenta colors.



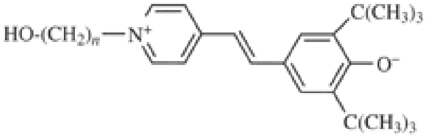
R = H **47**
R = CN **48**

For generation of cyan color, some squarylium dyes, eg, dye with azulene residues, can be used.

4.6. Photovoltaic and Solar Cells.

The photosensitivity of semiconductor electrodes used in photovoltaic cells is extended to longer wavelengths when typical polymethine dyes such as **15**, **16**, and **22** are used to spectrally sensitize the photoinduced separation of electrons and holes (1,7).

Photovoltaic cells that consist exclusively of a dye film deposited by evaporation in high vacuum between two metal electrodes have some advantages. Voltage generation in photovoltaic cells is improved when stilbazolium merocyanine dye molecules **49** ($n = 6$, 11) are ordered in a nematic liquid crystal that is sandwiched between two semiconducting (In_2O_3) electrodes.



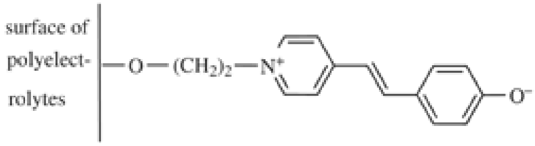
49

An additional improvement of solar cells is realized through combination of the cells with solar collectors. These are polymeric plates that contain any highly fluorescent PC. The sunlight absorbed by the dyes is converted to fluorescent light. The collected light is then converted to electric power by photovoltaic cells.

4.7. Probing Solvent Polarity and Hydrophobicity.

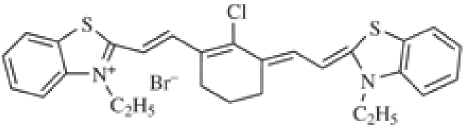
The absorption spectra of some merocyanines strongly depend on solvent polarity (*solvatochromy*). This has been used to develop several solvent polarity scales, eg, in terms of the parameter E_T.

When the merocyanine chromophore **50** is covalently attached via the *N*-alkyl group to polyelectrolytes, it enables not only the environment micropolarity, but also the electrostatic surface potential, to be probed spectroscopically (1,7).



50

The dimerization of dye **51** can be used to measure solvent hydrophobicity.

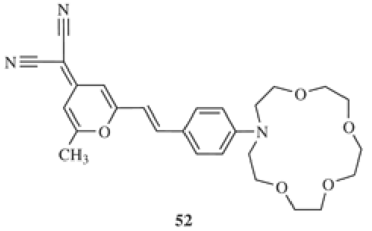


51

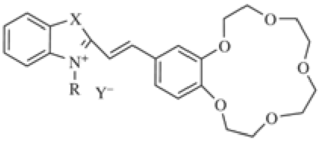
4.8. Ion Recognition. The complexing reagents most commonly used for cation detection are crown ethers linked with dye labels, the latter significantly changes their absorption and emission wavelengths as well as the fluorescence quantum yield, when the cations bind to the ethers (1,7).

A typical example of such *chromoionophores* and *fluoroionophores* is the merocyanine dye **52** which complexes with alkaline and alkaline-earth metal cations. The absorption spectrum of dye **52** undergoes strong changes on complexation, whereas the fluorescence spectrum is only slightly blue-shifted.

If a crown ether contains a styryl dye chromophore, as is the case with **53** [$X = C(CH_3)_2$, S; $R = CH_3$, CH_2CH_3, $(CH_2)_3SO_3^-\ M^+$], complexation with cations Mg^{2+} and Ca^{2+} promotes photoisomerization of the dye.

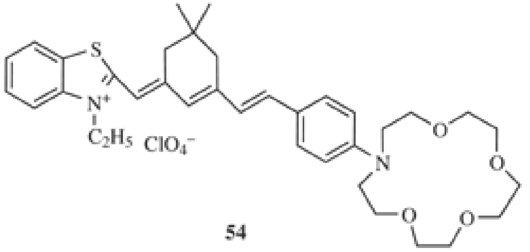


52



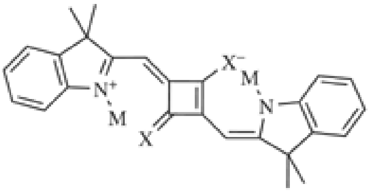
53

Polymethine dye **54** and its complexes with Li^+, Na^+, Ca^{2+}, and Sr^{2+} cations in acetonitrile solutions show absorption maxima at 574, 533, 513, 465, and 471 nm, and fluorescence maxima at 807, 793, 805, 786, and 791 nm, respectively.



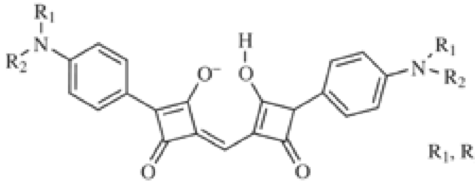
54

Recently, squarylium and thiosquarylium dyes were offered for spectrophotometric definition of metals. The complexes of squarylium and dithiosquarylium dyes with metals **55** and **56** show a striking selectivity for Cu^{2+} or Ag^{+} and are potentially suitable for practical sensor application.



M = metal ion
X = O **55**
X = S **56**

The complexation of bis(squarilium) dye **57** with transition metal cations such as Cr^{3+}, Mn^{2+}, Fe^{2+}, Co^{2+}, and Cu^{2+}, exclusively induces drastic absorption spectral changes with hypsochromic shifts ($\Delta\lambda = 59–197$ nm).



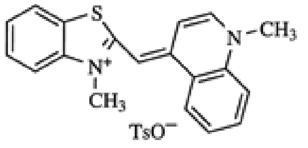
$R_1, R_2 = Alk$

57

4.9. Fluorescence Labeling in Molecular Biology.

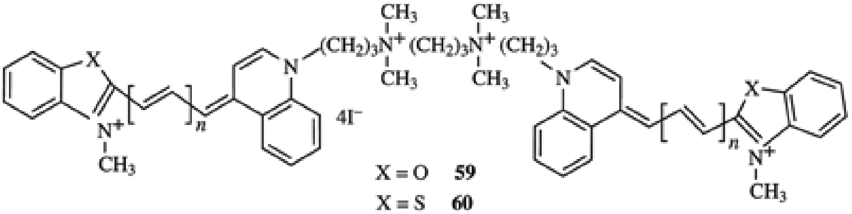
Fluorescence labeling is widely applied in the analytical detection of amino acids, proteins, nucleic acids, living cells, and in immunoassay techniques (1,7). Fluorescence labeling in molecular biology is realized both through the noncovalent interaction of dye chromophores with biomolecules and through covalent linkages.

Noncovalent fluorescence labeling of liposomes in living material (macrophages, lymphocytes, neurons, viruses, and bacteria) was realized with polymethine dyes **14** ($n = 1$, $R = C_{18}H_{37}$) and **15** ($n = 1$, $R = C_{18}H_{37}$). The liposomes were detected both spectrophotometrically, after their isolation from the living species, and directly by in vivo fluorescence microscopy. The monomethine dye Thiazole orange, **58**, has excellent properties as a biological noncovalent DNA and RNA label ($\lambda_{abs} = 509$ nm, $\lambda_{fl} = 525$ nm). This dye does not fluoresce until intercalated into DNA or RNA. After binding with DNA, it exhibits a fluorescence quantum yield of 0.2.



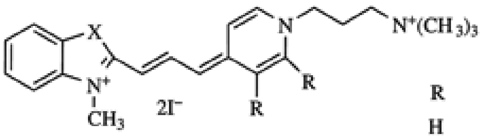
58

For the noncovalent labeling of double-stranded DNA and RNA, the lipophilic bis-intercalating dyes, **59** (YOYO - 1) ($n = 0$, $\lambda_{abs} = 491$ nm, $\lambda_{fl} = 508$ nm) and **60** (TOTO-1) ($n = 0$, $\lambda_{abs} = 514$ nm, $\lambda_{fl} = 533$ nm) are applied. On intercalation of the dyes among the nucleic acid double strands, their fluorescence intensity is enhanced by up to three orders of magnitude. The complexes are stable enough to allow the detection of prestained DNA restriction fragments after electrophoretic separation.



X = O **59**
X = S **60**

Trimethine unsymmetric monomeric cyanine dyes **61** (PO-PRO-3) ($\lambda_{abs} = 539$ nm, $\lambda_{fl} = 567$ nm), **62** (BO-PRO-3) ($\lambda_{abs} = 575$ nm, $\lambda_{fl} = 599$ nm), **63** (YO-PRO-3) ($\lambda_{abs} = 612$ nm, $\lambda_{fl} = 631$ nm), and **64** (TO-PRO-3) ($\lambda_{abs} = 642$ nm, $\lambda_{fl} = 661$ nm) are commercially available. The dyes have relatively narrow emission bands, thus allowing multicolor applications in imaging and flow cytometry.

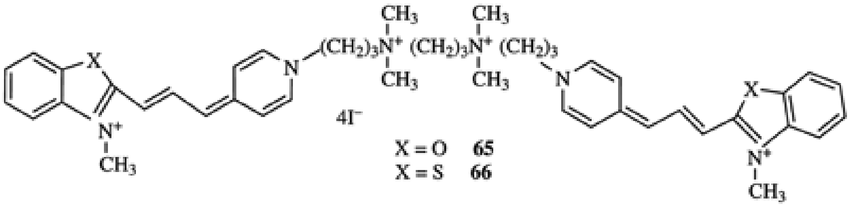


R	R	X	
H	H	X = O	**61**
H	H	X = S	**62**
–CH=CH–CH=CH–		X = O	**63**
–CH=CH–CH=CH–		X = S	**64**

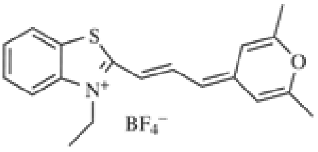
The trimethine unsymmetric homodimeric cyanine dyes **65** (POPO-3) ($\lambda_{abs} = 534$ nm, $\lambda_{fl} = 570$ nm), **66** (BOBO-3) ($\lambda_{abs} = 570$ nm, $\lambda_{fl} = 602$ nm), **59** ($N = 1$) (YOYO-3) ($\lambda_{abs} = 612$ nm, $\lambda_{fl} = 531$ nm), and **60** ($n = 1$) (TOTO-3) ($\lambda_{abs} = 642$ nm, $\lambda_{fl} = 660$ nm) bearing four positive charges have high binding affinity to nucleic acids and are also commercially available.

The development of cost effective and rapid methods for sequencing, interpreting, and storing DNA sequences for diagnostic applications, ranging from healthcare to agriculture and environmental monitoring, makes a very bright future for PDs as fluorescent probes for nucleic acid detection.

It was offered fluorescent labeling of biomolecules containing amino groups, based on interaction of them with pyrylocyanines, eg, with dye **67** ($\lambda_{max} = 536$, 572 nm), as a result of which are formed pyridocyanines ($\lambda_{max} = 540$ nm), covalently connected with biomolecules. The conjugates formed in this manner contain the pyridocyanine chromophores and have higher quantum yields of fluorescence.

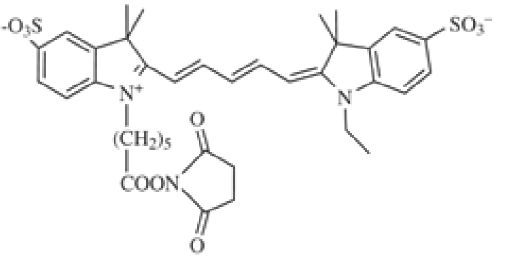


X = O **65**
X = S **66**



67

To improve water solubility and realize their covalent attachment to proteins, the substituted carboxylic group and two sulfonic acid groups were introduced into the heterocyclic rings of dye **68** (Cy5) ($\lambda_{abs} = 647$, $\lambda_{fl} = 664$ nm):



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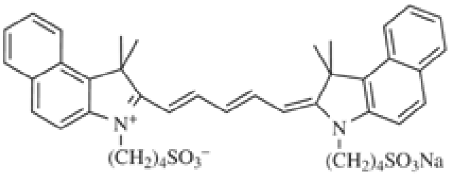
4.10. Application in Medicine. Polymethine dyes such as **14–16** ($n = 1,2$) are used for labeling of amino acids and in investigation of their transport in renal tissue, to visualize vasculature, and localize endoplasmic reticulum in living cells by fluorescence probing (1,7). Some polymethine dyes show antibacterial activity.

Indocyanine green, **69**, is used in medicine for diagnosis of cardiovascular illnesses and research on kidney and liver functions.



69

Dye **16** ($n = 2$) and some other polymethine dyes are effective anthelmintic means.

5. Conclusion

The polymethine dyes play a very important role in the development of the theory of color in organic dyes and spectroscopic theory. Their application in various areas of science and engineering constantly explained their properties the new and more and more high requirements. Therefore the chemistry of polymethine dyes gains new stimulus for the development of synthetic methods and the perfection of technology for the reception of dyes and the study of opportunities for their practical application. Technology for the application of polymethine dyes is also being perfected. For example, it was recently established that the single-layer light-emiting diod based on polyimide polymer and organic nanocrystalline particles (J-aggregates) of polymethine dyes gave a very narrow electroluminescent band in the near-ir region with $\lambda_{max} = 815$ nm.

BIBLIOGRAPHY

"Polymethine Dyes" in *ECT* 2nd ed., Vol. 16, pp. 282–300, by A. J. Cofrancesco and L. C. Hensley, GAF Corp.; in *ECT* 3rd ed., Vol. 18, pp. 848–874, by D. M. Sturmer and D. R. Diehl, Eastman Kodak Co.; in *ECT* 4th ed., Vol. 19, pp. 1004–1030, by Alexy D. Kachkovski, Institute of Organic Chemistry, National Academy of Sciences of the Ukraine; "Polymethine Dyes" in *ECT* (online), posting date: December 4, 2000, by Alexy D. Kachkovski, Institute of Organic Chemistry, National Academy of Sciences of the Ukraine.

CITED PUBLICATIONS

1. G. Bach and S. Daehne, *Cyanine Dyes and Related Compounds*, in *Rodd'*s Chemistry of Carbon Compounds, Vol. IV^{B}, *Heterocyclic Compound*, Sec. Suppl. 2nd ed., M. Sainsbury, Ed., Elsevier Science, Amsterdam, The Netherlands, 1997, Chap. 15, pp. 383–481.
2. N. Tyutyulkov, J. Fabian, A. Mehlhorn, F. Dietz, and A. Tadjer, *Polymethine Dyes: Structure and Properties*, St. Kliment Ohridski University Press, Sofia, Bulgaria, 1991.

3. A. A. Ishchenko, *Structure and Spectral—luminescent Properties of Polymethine Dyes*, Naukova Dumka, Kiev, Ukraine, 1994.
4. A. A. Ishchenko, *Usp. Khim.* **60**, 1708 (1991).
5. D. M. Sturmer, Syntheses and Properties of Cyanine and Related Dyes, in A. Weissberger, E. C. Teylor, eds., *The Chemistry of Heterocyclic Compounds*, Vol. 30. Wiley-Interscience, New York, 1977, pp. 441–587.
6. L. M. Yagupol'skii, A. Ya. Il'chenko, and L. Z. Gandel'sman, *Usp. Khim.* **52**, 1732 (1983).
7. S. Daehne, U. Resch-Gegner, O. S. Wolfbeis, Eds., *The Near-Infrared Dyes for High Technology Applications*, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1998.
8. A. I. Tolmachev, Yu. L. Slominskii, and A. A. Ishchenko, *New Cyanine Dyes Absorbing in the NIR Region*, in Ref. 4, pp. 385–415.
9. A. I. Kiprianov, *Usp. Khim.* **40**, 1283 (1971).
10. J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd ed., Kluver Academic/Plenum Publishers, New York, 1999.
11. A. Mishra, R. K. Behera, P. K. Behera, B. K. Mishra, and G. B. Behera, *Chem. Rev.* **100**, 1973 (2000).
12. B. I. Shapiro, *Usp. Khim.* **63**, 243 (1994).
13. K.-Y. Law, *Chem. Rev.* **93**, 449 (1993).
14. J. Fabian, H. Nakazumi, M. Matsuoka, *Chem. Rev.* **61**, 1197 (1992).
15. A. I. Tolmachev and A. Ya. Il'chenko, *Sci. Appl. Photo* **42**, 483 (2001).

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Table 1. **Spectral Characteristics of Dyes 2**

n	λ_{max} (CH_2Cl_2), nm	V, nm	lg
0	312.5		4.81
1	416	103.5	5.08
2	519	106	5.32
3	625	106	5.47
4	734.5	109.5	5.55

Table 2. **Correlation Equations for the Absorption Maximum Wavelengths, the Average Vinylene Shifts, and the End-Group Spectral Lengths**

Dye	Solvent	Correlation equation	Vinylene shift V, nm	End-group spectral length L_0, nm
2 ($n = 0$–4)	CH_2Cl_2	$\lambda_{max} = 310.8 + 105.3\,n$	105.3	129.1
3 ($n = 0$–3)	DMF[a]	$\lambda_{max} = 267.7 + 93.8\,n$	93.8	110.4
4 ($n = 0$–2, 4)	$(CH_3)_2SO$	$\lambda_{max} = 491.5 + 107.6\,n$	107.6	218.9
6 ($n = 1, 2$)	CH_2Cl_2	$\lambda_{max} = 668 + 95.0\,n$	95.0	310.3
7 ($n = 1, 2$)	CH_2Cl_2	$\lambda_{max} = 782 + 94.0\,n$	94.0	367.5
8 ($n = 0$–3)	CH_3COOH	$\lambda_{max} = 606.6 + 91.6\,n$	91.6	280.4
14 ($n = 1$–5)	C_2H_5OH	$\lambda_{max} = 436.2 + 103.6\,n$	103.6	192.2
15 ($n = 0$–3)	C_2H_5OH	$\lambda_{max} = 374.6 + 103.4\,n$	103.4	161.5
16 ($n = 1$–5)	C_2H_5OH	$\lambda_{max} = 448.4 + 106.6\,n$	106.6	197.6
17 ($n = 1$–3)	C_2H_5OH	$\lambda_{max} = 502.7 + 102.5\,n$	102.5	225.7
18 ($n = 1$–3)	CH_3OH	$\lambda_{max} = 587.3 + 114.0\,n$	114.0	265.0
19 ($n = 0$–3)	CH_2Cl_2	$\lambda_{max} = 559.1 + 122.1\,n$	122.1	249.0
20 ($n = 0$–3)	CH_2Cl_2	$\lambda_{max} = 630.7 + 129.7\,n$	129.7	282.9
21 ($n = 1$–3)	CH_2Cl_2	$\lambda_{max} = 654.0 + 113.0\,n$	113.0	298.8

[a] Dimethylformamide = DMF.