Isomerization and Properties of Isomers of Carbocyanine Dyes

Pavel Pronkin * and Alexander Tatikolov

N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Kosygin Str. 4, 119334 Moscow, Russia; tatikolov@mail.ru
* Correspondence: pronkinp@gmail.com; Tel.: +7-495-939-7171

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Abstract: One of the important features of polymethine (cyanine) dyes is isomerization around one of C–C bonds of the polymethine chain. In this review, spectral properties of the isomers, photoisomerization and thermal back isomerization of carbocyanine dyes, mostly meso-substituted carbocyanine dyes, are considered. meso-Alkyl-substituted thiacarbocyanine dyes are present in polar solvents mainly as cis isomers and, hence, exhibit no photoisomerization, whereas in nonpolar solvents, in which the dyes are in the trans form, photoisomerization takes place. In contrast, the meso-substituted dyes 3,3′-dimethyl-9-phenylthiacarbocyanine and 3,3′-diethyl-9-(2-hydroxy-4-methoxyphenyl)thiacarbocyanine occur as trans isomers and exhibit photoisomerization in both polar and nonpolar solvents. The behavior of these dyes may be explained by the fact that the phenyl ring of the substituent in their molecules can be twisted at some angle, removing the substituent from the plane of the molecule and reducing its steric effect on the conformation of the trans isomer. In some cases, photoisomerization of cis isomers of meso-substituted carbocyanine dyes is also observed (for some meso-alkyl-substituted dyes complexed with DNA and chondroitin-4-sulfate; for 3,3′-diethyl-9-methoxythiacarbocyanine in moderate polarity solvents). The cycle photoisomerization–thermal back isomerization of cyanine dyes can be used in various systems of information storage and deserves further investigation using modern research methods.

Keywords: carbocyanine dyes; isomerization; trans and cis isomers; meso-substituted cyanines

1. Introduction

Isomerization reactions, which involve rotation of a bulky molecular group around a body-fixed axis, often play a fundamental role in chemical and biological processes both in solution and in organized assemblies. In nature, the most important and known isomerization process is photoisomerization of 11-cis retinal, the chromophore of rhodopsin, which is a photoreceptive pigment for twilight vision [1,2]. Rotational isomerization is inherent in polyenic compounds, in particular, carotenoids and diphenylpolyenes [3]. Azo and azomethine dyes can also undergo cis-trans (or syn-anti) isomerization, while with more complex mechanisms [4,5]. The most abundant class of dyes that exhibit rotational isomerization is polymethine (cyanine) dyes.

At present, cyanine dyes attract great attention of researchers in different fields of science, technology engineering, pharmacology and medicine [6]. In particular, these dyes are widely used in laser technology (for creating tunable laser media in the near IR spectral range, passive Q-switches of lasers and other devices of quantum electronics and optoelectronics) [7,8]. Cyanine dyes also attract interest due to the prospects of their use as spectral-fluorescent probes and labels in the study of various biological systems [9–12], and in biomedicine for the purposes of visualization of tissues and photodynamic therapy [6,13–16]. This is due to the unique properties of cyanine dyes, which are
determined by the key features of their molecular structure. Various fields of applications of cyanine dyes determine the need for studying their photophysical and photochemical properties in solutions and molecularly organized media.

2. Structure and Spectral-Fluorescent Properties of Cyanine Dyes

Polymethine (cyanine) dyes contain conjugated polymethine chains (with an odd number of CH groups) with terminal nitrogen atoms. The nitrogen atoms can be a part of amino groups or terminal heterocycles B1 and B2 (quinolinyl, indolenyl, benzimidazolyl, benzothiazolyl, benzoxazolyl, and other heterocyclic residues, see Figure 1) [17,18].

![Figure 1](image1.png)

Figure 1. Generalized molecular structure of cyanine dyes and examples of terminal heterocycles B1 and B2.

The dye molecule usually carries a positive charge distributed over the polymethine chain. Due to complete \( \pi \)-electronic conjugation in the polymethine chain, these dyes have narrow absorption bands with high extinction coefficients (of the order of \( 10^5 \) L mol\(^{-1}\) cm\(^{-1}\)) in the spectral range from 340 to 1400 nm [6,9,17,18]. Note that symmetric polymethine dyes are characterized by equalization of C–C bonds in the chromophore and approaching of their orders to one-and-a-half (polymethine state). From symmetry considerations, it follows that for symmetric cyanines (B1 = B2) this should lead to a uniform distribution of the electronic density along the polymethine chain and identical charges on the terminal groups (Figure 2). Hence, the electronic structure of symmetric cationic dyes can be represented by a superposition of two resonance structures [17].

![Figure 2](image2.png)

Figure 2. Resonance structures of polymethine (cyanine) dyes.

Elongation of the polymethine chain in the symmetric dyes by one vinylene group results in a bathochromic shift of the absorption maximum by about 100–130 nm [17–19].

As a rule, in their absorption spectra there is only one short-wavelength vibronic maximum as a characteristic shoulder at the edge of the main band. The presence of a system of alternating charges determines the high sensitivity of polymethine dyes to intermolecular interactions with the medium.

Due to the lack of rigidity of the molecule, which is characteristic of cyanine dyes with an open polymethine chain, various vibrations and rotations of individual fragments are possible in their molecules, first of all, torsions and rotations around various bonds of the polymethine chain of cyanines. This also determines the possibility of isomerization due to rotation around the C–C bonds.
of the polymethine chain. Photoisomerization \((\text{trans} \rightarrow \text{cis} \text{ or } \text{cis} \rightarrow \text{trans})\) is a potent nonradiative channel for deactivation of the excited state of the molecules of polymethine dyes. This determines short lifetimes (from tens to hundreds of picoseconds) of their excited singlet state \((S_1)\), as well as low values of the quantum yields of fluorescence and intersystem crossing to the triplet state \((S_1 \rightarrow T)\) \([9,17,18]\). Hence, the study of photoisomerization of polymethine dyes is a key to understanding the photophysical and photochemical processes occurring in their molecules after photoexcitation.

At present, there is a wide variety of polymethine dyes: cationic dyes—monomethine cyanines, carbocyanines (or trimethine cyanines), dicarbocyanines (pentamethine cyanines), tricarbocyanines (heptamethine cyanines), etc., as well as anionic (in particular, oxonols) and uncharged dyes (in particular, merocyanines and ketocyanines). In this article, we confine ourselves to the most common class of polymethine dyes—carbocyanines with a positively charged chromophore. Note that the total charge of such dyes can be not only positive, but also negative (or neutral) due to the presence of anionic substituents.

3. Isomerization of Carbocyanine Dyes

As noted above, one of the main paths of degradation of the energy of the \(S_1\) state of cyanines in low-viscosity media is usually \(\text{trans} \rightarrow \text{cis} \text{ (or } \text{cis} \rightarrow \text{trans})\) isomerization, performed by rotating a fragment of the dye molecule around one of the bonds of the polymethine chain by \(\sim 180^\circ\) (also possible is internal conversion—the nonradiative transition \(S_1 \rightarrow S_0\) induced by vibrations and torsions). Figure 3 shows general molecular structures of the \text{trans} and \text{cis} isomers of the dyes.

![Generalized molecular structures of \text{trans} and \text{cis} isomers of unsubstituted carbocyanine dyes.](image)

The existence of photoisomers of polymethine dyes was first proven in 1965 by flash photolysis of dye solutions \([20]\). For example, for the unsubstituted cationic dye \(3,3'\)-dimethylthiocarbocyanine perchlorate (1), the photoisomerization quantum yield \((\phi)\) is 0.24 in dichloromethane \([21]\). Upon photoexcitation of its analogue, \(3,3'\)-diethylthiocarbocyanine iodide (2) in polar solvents (ethanol, dimethyl sulfoxide, acetonitrile), \(\phi\) has a similar value (0.25 in methanol \([22]\)); the contributions of fluorescence and intersystem crossing to the triplet state upon deactivation of excited singlet molecules are low.

Direct photoexcitation of carbocyanine dyes with no substituents in the polymethine chain (they are mainly present in solution in the form of \text{trans} isomers) leads to the formation of a short-lived \text{cis} photoisomer (see Equation (1)), which then undergoes back dark (thermal) isomerization to form the initial \text{trans} isomer (with a rate constant for \(2 k_i = 250 \text{ s}^{-1}\) in ethanol \([21]\)):

\[
k_i \quad \text{trans-}
\]

Upon flash photoexcitation of aerated solutions of the unsubstituted oxacarbocyanine dye \(3,3'\)-diethyloxocarbocyanine iodide (3) in isopropanol and aqueous phosphate buffer solution (pH 7, 20 mmol L\(^{-1}\)), the absorption and bleaching signals caused by photoisomerization and thermal back isomerization of the photoisomer formed was observed \([23]\).

The criterion determining the assignment of the signals to the photoisomer was the absence of the effect of oxygen on the observed signals, since the photoisomers of cyanine dyes are formed, as a rule, from the \(S_1\) state of the dyes having a too short lifetime (hundreds of picoseconds) to be quenched with atmospheric oxygen \([24]\). The bleaching maximum \((\lambda_{\text{max}} = 480 \text{ nm})\) of the difference spectra of 3 approximately coincides with the absorption band of the dye (\text{trans} isomer), and the absorption maxima \((\lambda_{\text{max}} = 505 \text{ nm})\) are in the long-wavelength region. The assignment of the
spectrum to the cis isomer is based on [25]. The value of \( k \) for 3 does not depend on the excitation and registration wavelengths and is 9.0 s\(^{-1}\) (in aqueous phosphate buffer solution) [23].

For the dyes that do not have substituents in the polymethylene chain, as a rule, the all-trans (EEEE) isomer is thermodynamically more stable. These dyes are mainly present in solution in the form of this isomer [26]. Their photoisomers correspond to a cis conformation [27].

4. Potential Surfaces of Isomerization of Cyanine Dyes

Historically, several models of potential surfaces of cyanine isomerization (surfaces corresponding to isomerization rotation around one of the C–C bonds of the polymethylene chain) were considered in the literature. One of the first models was the Rulliere model [28], in which the potential surfaces calculated by Orlandi and Siebrand for isomerization of stilbene [29] were mechanically transferred to the case of isomerization of cyanines. However, it was later shown [30] that the potential wave functions for polyenes and cyanines are of different nature, despite some similarity of the shapes of the potential surfaces for the \( S_1 \) state, so the results of Orlandi–Siebrand’s calculations are not applicable for cyanines.

On the basis of quantum-chemical calculations carried out for simple open-chain cyanine dyes (streptocyanines), two models of photoisomerization of cyanine dyes were proposed, taking into account the influence of solvent polarity in different ways [22]. According to these models, photoisomerization of cyanines occurs with activation energy barrier in the excited singlet state (\( S_1 \)) through the formation of an intermediate perpendicular (perp) form. The models also suggest an energy barrier for the reaction of thermal back isomerization of the dyes in the ground state (\( S_0 \)). According to model 1, photoisomers of thiacarbocyanine dyes are characterized by twisted, distorted configuration. This model assumes stabilization of intermediate perpendicular forms of dye photoisomers in polar solvents due to dipole–dipole interactions with the solvent [31]. Solvation of the perpendicular form should lead to a significant decrease in the corresponding energy barriers (minima on the \( S_1 \) and \( S_0 \) energy curves) [32]. In the absence of solvation of the perpendicular form, such effects are not expected [22,31]. In nonpolar solvents, a high energy barrier will prevent isomerization in the \( S_1 \) state. In accordance with model 1, the rate of the back isomerization reaction of photoisomers of cyanine dyes in the ground state should strongly depend on the solvent polarity [31,32].

Model 2 proposed by Momicchioli et al. [30] assumes the formation of planar cis isomers; dipole–dipole interactions with a solvent were not explicitly included in the calculations. The model assumes relatively small solvation effects on isomerization of cyanine dyes in polar media. In [22], the data on the photophysical properties and photoisomerization of dyes 2 and 3 in various solvents were analyzed.

The results obtained contradict the theoretical model of photoisomerization, in which the key role is played by the influence of solvent polarity (model 1) [22,31,32]. It was shown that, upon changing the dielectric constant of the solvent (\( \varepsilon = 2.2 \) to 32.7), no predicted effect of polarity on the \( S_1 \) state potential barrier in the photoisomerization reaction was observed. The absorption spectra of the photoisomers of dyes 2 and 3 correspond to the planar structures of the molecules, which do not correspond to model 1 [22]. Model 2 [29] is in better agreement with experimental data and allows one to take into account the weak effects of solvent polarity on the Arrhenius parameters of dye 2 back isomerization. For the trans-to-perp transition, the activation energy was found to be ~17–19 kJ/mol, the transition of the cis isomer to the perpendicular state (cis-to-perp) is apparently barrier-free and depends on the energy of the viscous flow of the solvent. For dark processes of back isomerization of the photoisomers, an energy barrier of ~54–75 kJ/mol is characteristic, depending on cyanine and solvent [22].

Now in the literature, a model of potential surfaces for cyanine dyes is used, starting from which, irrespective of the solvent, only the surface of \( S_1 \) has a third minimum (turn by ~90°), which roughly corresponds to the top of the potential barrier of the surface of \( S_0 \) having two minima (Figure 4) [30]. Upon photoexcitation, a molecule in the form of a trans isomer passes to the central minimum of \( S_0 \), from which, when it reaches the surface of \( S_0 \), it slips down to a minimum of cis-S0...
trans–cis photoisomerization) or trans-S\(_0\) (nonradiative degradation of the excitation energy). If the excited molecule is in the form of a cis isomer, then it also passes to the central minimum of S\(_1\), from which, when it reaches the surface of S\(_0\), it slips down to a minimum of cis-S\(_0\) (nonradiative degradation of the excitation energy) or trans-S\(_0\) (cis-trans photoisomerization). The quantum yields of photoisomerization and nonradiative degradation depend on the position of the minimum of S\(_1\) with respect to the maximum of S\(_0\). Theoretical calculations by the CS INDO method [30] and experimental verification of the consistency of this model with the isomerization process [22] showed its correspondence to cyanine isomerization.

![Figure 4](image)

**Figure 4.** General scheme of the potential surfaces of isomerization of cyanine dyes (only S\(_0\), S\(_1\), and T\(_1\) surfaces are shown).

For cyanines with no substituents in the polymethine chain, nonradiative relaxation of the excited singlet state (both trans- and cis-S\(_1\)), occurring by movement along the potential surface of isomerization, leads to both cis and trans isomers of the dye. Thus, for these dyes, photoisomerization occurs upon photoexcitation of both trans and cis isomers.

In the case of most meso-substituted thiacarbocyanines (first of all, alkyl-substituted), the minimum potential energy of the dye in the S\(_1\) state corresponding to the perpendicular conformation is shifted with respect to the maximum of the energy barrier of the S\(_0\) state toward the cis isomer (Figure 5) [22,30].

![Figure 5](image)

**Figure 5.** Scheme of the potential surfaces of isomerization of meso-substituted cyanine dyes (only S\(_0\), S\(_1\), and T\(_1\) surfaces are shown).
For this reason, upon nonradiative relaxation of the $S_1$ state of such dyes by moving along the $S_1$ potential surface, the trans conformation of the dyes is not attained and photoexcitation into the absorption band of the cis isomer does not lead to the formation of the trans form, that is, the cis isomers of most meso-substituted thiacarbocyanines are not photoisomerized [21].

4. Effect of Solvent Viscosity on the Kinetics of Photoisomerization and Back (Thermal) Isomerization

The kinetics of isomerization of cyanines in solution is determined, first of all, by a potential barrier of rotation around the isomerizing bond. In solutions, the character of molecular environment is also an important factor, which can have an effect on isomerization of cyanine dyes. For isomerization processes with relatively low activation energies close to the activation energy of the viscous flow of the solvent (this is usually isomerization from the $S_1$ state, that is, photoisomerization), it strongly depends on the viscosity of the medium (slows down sharply with increasing viscosity). Due to the very efficient photoisomerization process, the lifetime of the $S_1$ state of cyanines in low-viscosity solvents is much shorter than the radiative lifetime and is usually hundreds of picoseconds or less. In high viscosity media, internal rotations in the cyanine molecule are hindered, which leads to a drop in the quantum yield of photoisomerization and an increase in the quantum yield of fluorescence, the fluorescence lifetime, and the quantum yield of the triplet state, since fluorescence and intersystem crossing to the triplet state compete with photoisomerization [30].

The influence of viscosity on ultrafast processes of cyanine dye photoisomerization was studied in a number of works. In particular, in [33] the formation of short-lived photoisomers of 1,1'-diethyl-4,4'-cyanine (4), 2,2'-trimethinequinocyanine (5, Pinacyanol) was studied after excitation with picosecond laser pulses. It was shown that the yield of photoisomers of the dyes strongly depends on viscosity: the relative concentration of the photoisomer decreases with an increase in the viscosity of the solvent (a mixture of glycerol and methanol). Studies using ultrafast femtosecond spectroscopy of the process of photoisomerization of the cationic monometinecyanine dye 1,1'-diethyl-2,2'-cyanine (6) in alcohols are reported in [34]. A lower quantum yield for the photoisomer was observed in more viscous solvents. The increase in viscosity results in slowing down intramolecular vibrations and torsions in the photoexcited cyanine molecule, including rotations of fragments of the dye molecule around C–C bonds of the polymethine chain leading to photoisomerization.

In [35], the kinetics of isomerization of molecules of indocarbocyanine dyes with substituents in positions 3 and 3' of various length in solutions of alcohols (methanol–decanol series) was studied. The results indicate, in particular, that the nature of the reaction coordinate plays an important role in determining the dependence of photoisomerization on viscosity.

To take into account the effect of viscosity on the rate of the photoisomerization reaction, a number of theoretical approaches have been proposed. First of all, this is the approach based on the Kramers theory [36]:

\[
k = \frac{\omega_r \gamma}{4\pi \omega_b m} \left( \sqrt{1 + \left( \frac{2\omega_m}{\gamma} \right)^2} - 1 \right) e^{-\frac{E_a}{RT}}
\]

where $k$ is the rate constant, $\omega_b$ and $\omega_m$ are the frequency factors of the barrier on the potential energy surface in the vicinity of the reactants ($\omega_b$) and the top of the reaction barrier ($\omega_m$), respectively, $E_a$ is the activation energy of the reaction, and $R$ is the universal gas constant.

At high viscosity, Equation (2) turns into

\[
k = \frac{\omega_b \omega_r m}{2\pi m} e^{-\frac{E_a}{RT}}
\]
Under these conditions, the reaction rate constant should be inversely proportional to the viscosity of the solvent. However, for the isomerization reactions, this proportionality is usually not observed; it has been shown that the reaction rate constant with increasing solvent viscosity decreases much slower than Equation (3) predicts [37,38].

An explanation of the dependence of the isomerization rate constant on viscosity was also proposed in the theory of Grote and Hynes [39], which introduces the concept of frequency-dependent friction affecting the rate of chemical processes in the solvent phase. Due to the inclusion of frequency-dependent friction instead of constant friction, the theory of Grote and Hynes successfully predicts the rate constant of chemical reactions in viscous liquids.

A more detailed consideration of theoretical approaches describing the dependence of the isomerization kinetics on viscosity is beyond the scope of this review.

For isomerization processes, the following semi-empirical equation is also used [40–42]:

$$E_a = E_0 + \alpha E_\eta$$  \hspace{1cm} (4)

where $E_a$ is the apparent activation energy of isomerization, $E_0$ is the internal energy barrier of isomerization, $E_\eta$ is the activation energy of a viscous flow, and $\alpha$ is a coefficient depending on the slope of the potential barrier in the transient state of the process ($0 \leq \alpha \leq 1$). For photoisomerization, the coefficient $\alpha$ is larger than for the thermal back isomerization (in the latter case, a weak dependence of $E_a$ on the viscosity is observed, which is manifested only in highly viscous solvents).

In [42], for photoisomerization of dye 3 in different alcohols the values of $\alpha$ were found to be in the range of 0.54 to 0.76, and for thermal back isomerization $\alpha \sim 0.27–0.41$.

5. Effects of Ion Pair Formation on the Isomerization Processes of Cyanine Dyes

In polar solvents, cyanine dyes are present in the form of free ions (cations); in this case, the influence of polarity of the medium on the isomerization of polymethine dyes is small (see above) [22,42]. However, in weakly polar and nonpolar solvents, the dyes form ion pairs with a counterion, which can affect their photochemistry, in particular, the processes of photoisomerization and back isomerization of the resulting photoisomer.

In [43], photophysical and photochemical processes occurring upon flash photoexcitation in molecules of dyes 1 (iodide) and 2 (chloride) in a mixture of dimethyl sulfoxide (DMSO)–toluene of various compositions were studied. With a decrease in the DMSO content in the solution down to 3 vol %, the relative quantum yield of intersystem crossing of dye 1 to the triplet state increases by two orders of magnitude, which is accompanied by a decrease in the quantum yield of trans–cis photoisomerization. This effect is not observed for dye 2. The authors explain these results by the heavy atom effect upon the formation of ion pairs of the dye cation 1 with the iodide counterion under nonpolar conditions.

In [44], the effect of counterions (ClO$_4^-$, Cl$^-$, I$^-$) on photochemical processes (including isomerization) in molecules of cationic benzimidacyanine dyes was studied in acetonitrile–toluene mixtures. In low polarity media, in which the dyes formed ion pairs with counterions, an increase in the rate constant and a decrease in the activation energy of back isomerization of benzimidacyanines were observed. The effect was explained by the electrostatic influence of the counterion in ion pairs on the electronic density distribution in the dye cation, leading to a decrease in the activation barrier of the process [44]. The influence of the counterion on the transient (“twisted”) state of isomerization, which lowers its energy, is also possible.

6. Isomerization of Meso-Substituted Carboxyamine Dyes

While carboxyamine dyes, which do not have meso substituents in the polymethine chain, are present in solutions as trans isomers, meso-substituted thiacarboxyamine dyes in the crystalline state are usually present as cis isomers [45].

Bulky substituents in the meso (9) position of the polymethine chain create significant steric hindrances to the formation of the trans isomer (Figure 6). At low temperatures in solutions, the absorption bands of meso-substituted thiacarbocyanines are split into two closely spaced bands [46].
Simultaneous presence (in comparable concentrations) of all-trans and cis isomers can be observed [45,46]. In particular, it has been shown that 3,3′,9-trimethylthiacarbocyanine iodide (7; Cyan 2), having a meso-methyl substituent in the polymethylene chain, is mainly in the cis form in polar solvents [47]. The equilibrium position depends strongly on the solvent polarity: the trans isomer predominates in low polarity media, while in highly polar solvents the trans isomer presents only as a small impurity to the cis isomer [26,45–48]. As an example, it was shown that 3,3′-diethyl-9-methoxythiacarbocyanine 8 in solvents of moderate polarity (ethyl acetate, isopropanol) is in the form of an equilibrium mixture of cis and trans isomers (Figure 7). The meso-substituted carbocyanine dye 3,3′,9-triethylthiacarbocyanine iodide (9) in isopropanol is in the form of a cis isomer, and in dioxane in the form of a trans isomer [49]. It was shown that 3,3′-diethyl-9-chlorothiacarbocyanine perchlorate (dye 10) is in the form of a cis isomer in water, and in the form of a trans isomer in a less polar solvent, isopropanol [50].

Figure 6. Structures of trans and cis isomers of meso-substituted carbocyanine dyes.

Figure 7. Absorption spectra of dye 8 (3,3′-diethyl-9-methoxythiacarbocyanine iodide; c_{dy} = 1.1 \times 10^{-6} \text{ mol L}^{-1}) in (1) acetonitrile, (2) isopropanol, (3) mixture of isopropanol and dioxane (1:1), and (4) dioxane.

An increase in the effective volume of the substituent in the meso position of the polymethylene chain of the molecule promotes an increase in the cis isomer content. It was established that 9-tert-butyl-3,3′-diethylthiacarbocyanine cation (11) exists both in solution and in the solid phase exclusively as a nonplanar di-cis (EZZE) form [26,47].

The simultaneous presence of trans and cis isomers in dye solutions is explained by distortion of the planar structure of the dye made by substituents in the meso position of the polymethylene chain of the dyes. This leads to a significant increase in the energy of the trans isomer, which becomes close to that of the cis isomer [45,46,51]. The observed dynamic equilibrium depending on the solvent polarity is due to the electrostatic interaction of the dye cation with a counterion in ion pairs formed in low polarity media. The formation of ion pairs in low polarity media leads to a decrease in the
energy of the trans isomer and its preferential formation, whereas in polar media the cis isomer is formed, which has a lower energy.

The different isomers present in the solution cause a difference in the photochemical properties of meso-substituted carbocyanine dyes in solvents of different polarity. In polar solvents, where the dyes are predominantly in the form of cis isomers, photoisomerization is generally not observed, while in low polarity solvents, in which the dyes are present as trans isomers, photoisomerization to form cis isomers occurs upon photoexcitation. This empirical rule is fulfilled by a series of thia- and oxacarbocyanines with bulky meso-substituents.

Upon flash photoexcitation of the carbocyanine dye 9, the signals corresponding to photoisomerization processes were not detected in the polar media (isopropanol), i.e., the cis isomer of 9 was not photoisomerized. In nonpolar dioxane solution, the signals corresponding to trans–cis photoisomerization and subsequent decay of the cis photoisomer of dye 9 were detected [50]. The thiacarbocyanine dye 10, which has Cl as a substituent in the meso-position of the polymethine chain, forms cis isomer by trans–cis photosomerization upon flash photolysis in isopropanol solution [50].

For meso-substituted oxacarbocyanine dyes 3,3′-dimethyl-9-ethylxocarbocyanine (12) and 3,3′,9-triethyl-5,5′-dimethylxocarbocyanine (13), the formation of cis photoisomers from their trans forms in aqueous solutions is also detected upon flash photoexcitation. The intensity of the signals of photoisomers of 12 and 13 is much lower than that for their unsubstituted analogue 3, being in the trans form, which can be explained by the presence of a significant admixture of cis isomers of the dyes (in equilibrium with trans isomers), whose photoisomerization does not occur [45,46].

It should be noted that the empirical rule under consideration also found exceptions. First of all, these include the case of two meso-substituted thiacarbocyanine dyes, 3,3′-dimethyl-9-phenylthiacarbocyanine iodide (14) and its analogue, 3,3′-diethyl-9-(2-hydroxy-4-methoxyphenyl)thiacarbocyanine iodide (15). For these dyes, the simple empirical rule that we are considering here is not satisfied. The analysis of the absorption, fluorescence, and fluorescence excitation spectra has shown that dyes 14 and 15 in solutions, regardless of the polarity of the solvent, are in the form of trans isomers [52]. Photoisomerization is observed for these dyes even in polar solvents. In particular, upon flash photoexcitation of 14 and 15 in ethanol, the difference spectra of cis photoisomers are observed, the bleaching maxima of which (\(\lambda_m\) = 560 and 568 nm, respectively) approximately coincide with the positions of the trans isomers in the stationary absorption spectra of the dyes. As in the case of meso-unsubstituted dye 2, which forms cis photoisomer upon photoexcitation [21,25], the stationary absorption spectra of the photoisomers of dyes 14 and 15 are shifted to the short-wavelength side (\(\lambda_{max} \approx 550\) and 558 nm, respectively).

Apparently, for dyes 14 and 15, the substituents in the meso position of the polymethine chain introduce less steric distortion into the structures of the trans isomers than alkyl substituents in the meso-alkyl-substituted dyes [52]. This is probably explained by twisting of the phenyl meso substituents in 14 and 15 relative to the dye chromophore plane, which sharply reduces their distorting effect on the planar structure of the dye chromophore and, hence, on the relative energy of the isomers.

Furthermore, for some meso-substituted thiacarbocyanines, photoisomerization of both trans and cis isomers is observed. As an example, dye 8 may be considered, for which both trans \(\rightarrow\) cis and cis \(\rightarrow\) trans photoisomerization processes were detected. In solvents of moderate polarity (ethyl acetate, isopropanol), depending on the photoexcitation wavelength, photoisomerization of either cis or trans isomer of 8 was observed, leading to the formation of trans or cis photoisomer, respectively [50]. The rate constants of thermal cis–trans transitions for ethyl acetate and isopropanol solution of 8 are given in Table 1. Since the cis and trans isomers in the solution of dye 8 are in equilibrium, the observed rate constant of the signal decay corresponds to the sum of the rate constants of cis \(\rightarrow\) trans and trans \(\rightarrow\) cis isomerization.

The rate constants of thermal cis–trans transitions for most meso-substituted thiacarbocyanine dyes were found to be much higher than for their unsubstituted analogs (see Table 1) [21,23]. Such an increase in the rate constants of the thermal back isomerization is due to steric hindrances, which create bulky groups of substituents in the meso position of the polymethine chain. The steric
influence of the *meso* substituents leads to removing terminal heterocyclic groups of dye molecules from the plane, which results in distortion of the valence angles, an increase in the energy of the *cis* and *trans* isomers, and a corresponding decrease in the potential barrier of thermal isomerization in the ground state [21].

<table>
<thead>
<tr>
<th>Dye</th>
<th>R</th>
<th>Solvent</th>
<th>$k_b$, s$^{-1}$</th>
<th>Ref.</th>
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<td>Thiacarbocyanine dyes</td>
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<tr>
<td>2</td>
<td>–</td>
<td>ethanol</td>
<td>250</td>
<td>[21]</td>
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<tr>
<td>8</td>
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<td>isopropanol</td>
<td>$1.7 \times 10^6$</td>
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<tr>
<td>9</td>
<td>C$_2$H$_5$</td>
<td>dioxane</td>
<td>$-5 \times 10^3$</td>
<td>[50]</td>
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<tr>
<td>10</td>
<td>Cl</td>
<td>isopropanol</td>
<td>$1.6 \times 10^8$</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>C$_2$H$_5$</td>
<td>ethanol</td>
<td>340</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>C$_6$H$_5$OH</td>
<td></td>
<td>865</td>
<td>[52]</td>
</tr>
<tr>
<td>22</td>
<td>S$_2$CH$_3$</td>
<td>isopropanol</td>
<td>$5 \times 10^3$</td>
<td>[58]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxacarbocyanine dyes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>–</td>
<td></td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>C$_2$H$_5$</td>
<td>aqueous solutions</td>
<td>$1.5 \times 10^4$</td>
<td>[23]</td>
</tr>
<tr>
<td>12</td>
<td>C$_2$H$_5$</td>
<td></td>
<td>$1 \times 10^4$</td>
<td></td>
</tr>
</tbody>
</table>

The observed rate constants for the decay of *cis* photoisomers of 14 and 15 were rather close to $k_b$ for the unsubstituted dye 2 (see Table 1), indicating a weak steric effect of *meso* substituents in 14 and 15 on the configuration of the *cis* photoisomer [52]. This also can be explained by the possibility of turning the phenyl ring of the substituent, which removes the substituent from the chromophore’s plane of the molecule and reduces its steric effect on the configuration of the isomers.

7. Isomerization of Cyanine Dyes in Structurally Organized Media

It is known that polymethine dyes can form noncovalent complexes with surfactants, polyelectrolytes, biomacromolecules [6,9,10,14,15,18,53–56]. The formation of such complexes, as a rule, hinders the intramolecular degrees of freedom of dye molecules leading to nonradiative dissipation of excitation energy, which results in a drop in the quantum yield of photoisomerization and an increase in the quantum yield of fluorescence [6,9,10,53]. In the case of *meso*-substituted polymethine dyes, which are characterized by dynamic *cis–trans* equilibrium depending on the medium, the formation of such complexes can significantly affect the *cis–trans* equilibrium.

In [54], photophysical and photochemical processes occurring in molecules of *meso*-substituted thiacarbocyanine dyes 3,3′-bis(γ-sulphopropyl)-5-methoxy-4,5′-benzo-9-ethylthiacarbocyanine (16), 3,3′-bis(γ-sulphopropyl)-6,6′-dimethyl-9-ethylthiacarbocyanine (17), and 3,3′-bis(γ-sulphopropyl)-5-methyl-5′-phenyl-9-ethylthiacarbocyanine (18) in aqueous solutions in the presence of cationic (cetyltrimethylammonium bromide), anionic (sodium dodecyl sulfate, SDS) and neutral (Triton X-100) surfactants were studied.

In an aqueous solution in the absence of surfactants, dyes 16–18 are in the form of dimers in equilibrium with *cis* monomers and are not capable of photoisomerization. At surfactant concentrations above critical micelle concentration (CMC), the dimers decompose into monomers, and *cis* monomers are converted into the *trans* form upon solubilization of the dye molecules in the surfactant micelles. The resulting *trans* isomers of the dyes are capable of photoisomerization to form *cis* photoisomers.

The behavior of *meso*-substituted polymethine dyes 3,3′-di-(γ-sulphopropyl)-4,5,4′,5′-dibeno-9-ethylthiacarbocyanine betaine (19, DEC) and 5 was studied in micellar systems of biological surfactants—bile salts sodium cholate, sodium deoxycholate, and sodium taurocholate [55]. Upon binding to micelles of bile salts, as in the case of synthetic surfactants, these dyes are converted into a *trans* form. Upon flash photolysis of aerated aqueous solutions of the dyes in the presence of micelles of bile salts and SDS, signals caused by photoisomerization of the *trans* isomers of the dyes and thermal back isomerization of the *cis*
photoisomers formed were observed. The lifetimes of photoisomers of 7 and 19 in the presence of bile salt micelles were in the range of 60–190 μs. In deoxygenated solutions, the appearance of the triplet state of the dyes was also observed.

In [56], photophysical and photochemical properties of thiacarbocyanine dyes 2, 3,3′-diethyl-9-methylthiacarbocyanine iodide (20) and 3,3′,9-triethyl-5,5′-dichlorothiacarbocyanine iodide (21) were studied in the presence of water-soluble polyelectrolytes: polystyrene sulfonate (PSS), polyacrylic acid (PAA), and polymethacrylic acid (PMA). For 2 in the presence of PSS, a significant (tenfold) decrease in the quantum yield of trans–cis photoisomerization (accompanied by an increase in fluorescence and a moderate increase in the intersystem crossing to the triplet state) was observed.

Besides surfactants and synthetic polyanions, cationic cyanine dyes are capable of forming noncovalent complexes with DNA. The interaction with DNA hampers the formation of photoisomers of meso-unsubstituted polymethine dyes. In [57], a drop in the yield of the photoisomer of dye 2 was observed upon the formation of its noncovalent complex with ds-DNA in a buffer solution. Upon flash photolysis of aerated solutions of meso-substituted thiacarbocyanine dyes 9, 10, 14, 15 and 3,3′-diethyl-9-thiomethylthiacarbocyanine iodide (dye 22) in the presence of DNA (with DNA concentration in the range of (2.3–5.0) × 10^{-4} mol L^{-1}), signals of photoisomers were not observed [50,52,58]. Photoisomerization was also hampered by noncovalent interaction of oxacarbocyanine dyes 3, 12, 13 with DNA [23]. Upon flash photolysis of aerated solutions of 12 and 13 with DNA at c_{DNA} = 4.4 × 10^{-5} mol L^{-1} and higher, the signals of photoisomers were not observed. For dye 3 with c_{DNA} = 2.39 × 10^{-4} mol L^{-1}, the absorption intensity of the photoisomer decreases 2.5 times, and its lifetime increases 5 times (Figure 8).

**Figure 8.** (1) Absorption spectrum of oxacarbocyanine dye 3 (c_{Dye} = 1.17 × 10^{-4} mol L^{-1}) in a phosphate buffer solution in the presence of DNA (c_{DNA} = 7.5 × 10^{-5} mol L^{-1}), (2) difference absorption spectrum of the dye photoisomer (c_{Dye} = 6.2 × 10^{-7} mol L^{-1}) obtained by flash photolysis in the presence of DNA, and (3) reconstruction of the photoisomer absorption spectrum. Inset: dependence of the decay kinetics of the photoisomer of dye 3 corresponding to back dark isomerization on the relative concentration of DNA.

Thus, the interaction with DNA and synthetic polyanions hampers the formation of photoisomers in the case of a large number of cationic polymethine dyes, which is explained by the steric factor of complexation, which slows down the nonradiative processes of dissipation of excitation energy. Nevertheless, for some meso-substituted thiacarbocyanine dyes, the formation of photoisomers was enhanced upon the complexation. In particular, in [59], the photophysical and photochemical properties of the meso-methyl-substituted dye 20 were studied in aqueous solutions.
in the presence of PSS, PAA and PMA. Flash photolysis of 20 in the presence of PAA and PMA revealed a weak signal due to cis $\rightarrow$ trans photoisomerization of the dye bound to the polyelectrolyte in the cis form and subsequent dark decay of the resulting trans photoisomer ($\tau = 700 \mu s$ at 560 nm). This contradicts the rule that cis isomers of meso-alkyl-substituted carbocyanines are not photoisomerized (dye 20 is not photoisomerized in an aqueous solution without polyelectrolytes). Similarly, in [47], an enhancement of cis $\rightarrow$ trans photoisomerization of meso-methyl-substituted dyes 7 and 20 by noncovalent binding to DNA and chondroitin-4-sulfate in a buffer solution was shown. Upon flash photolysis of 7 and 20 in the presence of DNA and chondroitin-4-sulfate, signals due to the formation of trans photoisomers and their subsequent dark decay ($\tau = 3.9$ and 2.2 ms for 7 in the presence of DNA and chondroitin-4-sulfate, respectively; $\tau = 0.7$ ms for 20 in the presence of biopolymers, $\lambda_{\text{em}} = 560$ nm) were observed. To explain the effect of stimulation of photoisomerization of meso-substituted cis isomers by complexation with polyelectrolytes, an assumption was made that the biopolymer matrix of polyelectrolyte affects the potential surfaces of photoisomerization of the dyes: deformation of the potential surface of $S_i$ occurs with a shift of its minimum toward the trans isomer [47,59].

8. Conclusions

The use of polymethine dyes in various fields of science and technology requires a detailed study of their photophysical and photochemical properties. Recently, a lot of works have been published on photophysics and photochemistry of polymethylene dyes (see, for example, [11,12,14–16,60–62]). Photoisomerization (followed by back isomerization of a photoisomer) is one of the main properties of polymethine dyes; at present, it is being studied in detail with the use of modern micro-, nano-, pico- and femtosecond techniques. While trans–cis (photo)isomerization of various compounds is widely used in molecular photoswitches, over the past decades, most works have been focused mainly on azobenzene derivatives [63], spiropyans [64], diarylethenes [65], and stilbenes [66], and, until recently, this property of cyanine dyes has not been applied in practice. The use of cyanine photoisomerization in research is mainly for probing microviscosity of microheterogeneous media [67,68]. On the contrary, rigidization of the polymethylene chain of dyes is often carried out by introducing “bridges” that suppress internal conversion and photoisomerization processes to increase the stability of the dyes and the fluorescence quantum yield [17,18,69,70]. It should be noted that the cycle photoisomerization–reverse dark isomerization of the photoisomer is completely reversible and, ideally, does not lead to decomposition of the dye molecule. Since the lifetime of the photoisomer, depending on the structure of the dye and the medium, can vary in very wide range (from seconds to picoseconds), this cycle can be used for transient recording and storage of information within different time ranges in modern information recording systems and devices. So we may expect in future widespread use of polymethylene dyes as information storage media in various fields of science and technology.

Of great importance is also the dynamic cis–trans isomeric equilibrium, which strongly depends on the molecular microenvironment, observed for meso-substituted polymethine dyes. Since the photophysical and photochemical properties of the cis and trans isomers of such dyes are very different, these dyes can be widely used as probes for studying various molecularly organized and nanostructured systems. Indeed, a number of meso-substituted cyanine dyes have already been used as spectral-fluorescent probes for biomacromolecules (DNA, albumins, etc.) [71–77]. Next in turn is the use of polymethylene dyes as kinetic probes, as it has been found recently that the lifetime of the photoisomers of meso-substituted oxonols (anionic polymethylene dyes) [78] and squarylium cyanines [79] depend strongly on the polarity of the molecular microenvironment.

In summary, it can be noted that the study of (photo)isomerization and the properties of isomers of cyanine dyes using modern research methods is still relevant both for theory and for the practical application of cyanine dyes.

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