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Chapter

Photophysical Properties of 4-(Dicyanomethylene)-2-Methyl-6-(4-Dimethylaminostyryl)-4*H*-Pyran (DCM) and Optical Sensing Applications

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Abstract

4-(Dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran (DCM) is, commonly known as red dye, an electron donor-acceptor molecule that exhibits very interesting photophysical properties such as high molar absorption coefficients, tunable electronic absorption and fluorescence emission energies, and high fluorescence quantum yields. Several DCM analogous have been synthesized and explored for various practical applications that include solid-state lasers, organic light-emitting diode (OLED), fluorescent sensors, logic gates, photovoltaics, nonlinear optics (NLO), and bioimaging of cells. In recent years, a significant amount of research work has been devoted for developing optical sensors based on DCM dye for detection of various guest analytes. The first part of this book chapter describes comprehensive photophysical properties of the DCM dye which include the results of steady-state and time-resolved absorption and fluorescence studies. The second part of the book chapter summarizes the recent developments of DCM-based optical sensors that exhibit colorimetric, ratiometric, and fluorosensing towards selective detection of metal cations, anions, and neutral species.

Keywords: red dye, electron donor-acceptor molecules, photophysical properties, optical sensors, NIR fluorescence, dicyanomethylene-4H-benzopyran, dicyanomethylene-4H-pyran, chemosensors

1. Introduction

The molecule, 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)-4*H*-pyran (DCM) belongs to the merocyanine dye category and is well-known in the literature as red fluorescent dye. The DCM dye consists of N,N-dimethylaniline group, electron donor and dicyanomethylene, and electron acceptor which are covalently attached by a π-conjugated moiety, 4*H*-pyran-4-ylidiene, in the form of electron donor-acceptor (D-π-A) architecture. The DCM was first reported by Eastman Kodak Company and initially used as dopant in developing red laser

materials [1]. However, in subsequent years it was found that DCM exhibit high fluorescence quantum efficiency, large Stokes shift, and solvatochromic behavior. Further, the absorption spectrum of DCM dye has minimum overlap with its fluorescence spectrum which was utilized in lasing action, for developing red lasers, and organic light-emitting diode (OLED) materials [2, 3]. Because of their interesting photophysical and optoelectronic properties, several research groups actively involved in developing DCM analogues not only for OLED application but also for logic gates, lasers, bioimaging, sensors, photovoltaics, and NLO applications. Way back in 2004, Chen reviewed how red emitting DCM derivatives have evolved as dopants for OLED device applications [4]. Later in 2012, Tian has published one review article which describes not only OLED applications of DCM-type materials but also fluorescent sensors, logic gates, photovoltaic sensitizers, nonlinear optical materials, bioimaging dyes, etc. [5]. Considering simple synthetic procedures of DCM derivatives [6], many optical sensors were reported based on DCM derivatives for recognizing various guest analytes, and the number of publications is rapidly increasing day by day. However, on the other hand, a comprehensive summery of DCM photophysical behavior has not been reported till date. Moreover, to the best of our knowledge, there is no single report that describes optical sensing behavior of DCM and its derivatives. The book chapter describes both the fundamental photophysics of DCM and recent progress on DCM derivatives as optical sensors.

2. Photophysical properties of DCM

2.1 Absorption

Electronic absorption spectrum of DCM in polar medium, dimethylsulphoxide (DMSO) was reported for the first time by Hammond in 1979 [7]. Later on, the DCM dye absorption spectra in different medium were studied in various contexts, and it is observed that the electronic absorption behavior is quite similar to many charge transfer (CT) dyes [7–16]. The DCM dye has very broad absorption, typically in between 200 and 600 nm, and the nature of the absorption strongly depends upon polarity of the medium (**Figure 1** and **Table 1**) [11, 12, 15, 16]. The DCM exhibits two absorption bands where the longer-wavelength band is found to be more intense than the shorter-wavelength band. In non-polar solvents, the shape of the longer-wavelength band is found to be more structured (vibronic structure) like any other CT dye molecules, and in polar solvents the structured nature disappears [17]. For example, the electronic absorption spectrum of DCM in cyclohexane shows two bands: structured longer-wavelength band with a maximum at 451 nm and shoulder at 340 nm [11]. However, when the same DCM dye is present in highly polar solvent like DMSO, a structureless longer-wavelength band is observed with maxima at 482 nm with a shoulder at 350 nm. Interestingly, the electronic absorption maximum of DCM undergoes a redshift upon increasing with the polarity of the medium which is commonly known as solvatochromic shift of the absorption. The solvatochromic behavior is more prominent in polar aprotic solvents than that of polar protic solvents with that of non-polar solvents. For example, the absorption maxima of DCM in cyclohexane and DMSO in the solvatochromic shift is found to be ~30 nm, which is relatively less (~20 nm) when compared to cyclohexane and ethanol absorption maxima. From the Lippert-Mataga theory [18–20], ground-state dipole moment (μ_a) of the DCM was estimated to be 5.6 D which suggests that the DCM is a dipolar molecule [11]. It is well-known in the literature that an electronic state of a dipolar molecule is more stabilized in polar solvents rather than in less polar or non-polar solvents. So, the observed solvatochromic behavior in different solvents

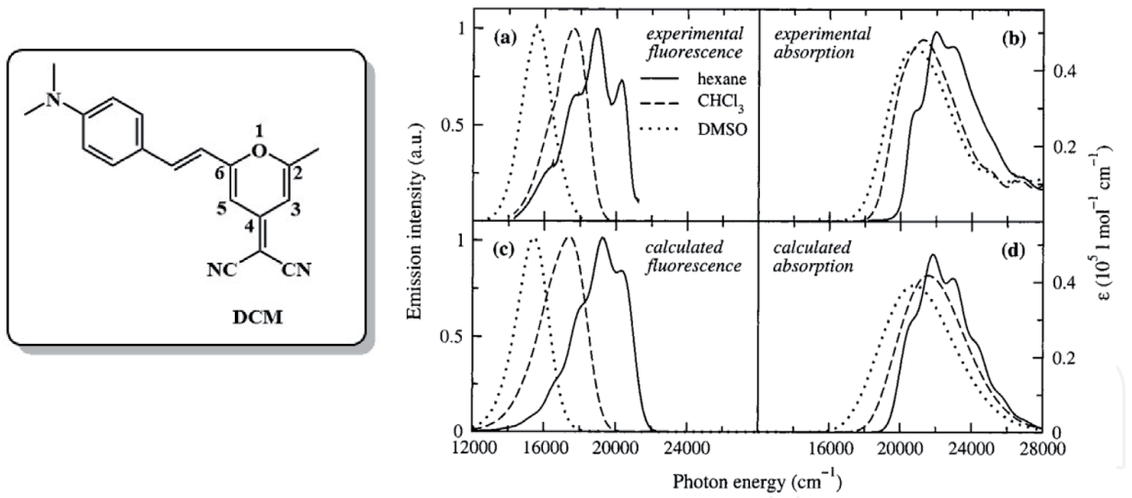

Figure 1.
Molecular structure of DCM (left). Right side, experimental (a) fluorescence and (b) absorption spectra of DCM dissolved in hexane (continuous lines), $CHCl_3$ (dashed lines), and DMSO (dotted lines) and (c) fluorescence and (d) calculated absorption spectra. Reproduced with permission from ACS [16].

Sl. no	Solvent	λ_a (nm)	λ_f (nm)	$\lambda_a - \lambda_f$ (nm)	ϕ_f	τ (ns)
1	n-Hexane	451	530	79	0.05	0.015
2	Cyclohexane	454	533	79	—	—
3	1,4-Dioxane	456	566	100	—	—
4	Toluene	461	567	106	0.08	0.022
5	Chloroform	471	565	94	0.35	—
6	Tetrahydrofuran	—	—	—	0.49	1.24
7	Dichloromethane	468	587	109	—	—
8	Acetonitrile	463	617	154	0.45	1.95
9	DMF	475	626	151	—	—
10	DMSO	481	644	163	0.80	2.25
11	Methanol	466	623	157	0.3	1.36
12	EtOH	470	614	144	—	—
13	n-Propanol	472	614	142	0.57	2.10
14	PMMA	453	550	97	0.76	2.00

λ_a, absorbance maxima (nm); λ_f, fluorescence emission maxima; $\lambda_a - \lambda_f$, Stokes shift; τ, fluorescence lifetime; ϕ_f, fluorescence quantum yield.

Table 1.
Photophysical parameters DCM [7–16].

is attributed to the extent of dipole–dipole interactions in the respective solvents. Dipole–dipole interactions are prominent in polar solvents and aromatic solvents, and corresponding energy state will be relatively more stabilized; thereby a red-shift of the absorption maxima is quite obvious. Similarly, the structured longer-wavelength absorption band, observed in non-polar solvents, is primarily due to the vibronic coupling where the vibrational energy levels are well separated and thus their vibrational transitions become prominent. The vibronic coupling is even more prominent at the low-temperature (77 K) experiments and can be attributed to the absence of dipole–dipole interactions [15]. Molar absorption coefficients of DCM in

ethanol are estimated to be 4.2×10^4 mol^{-1} cm^{-1} at its absorption maxima (470 nm) and 1.2×10^4 $mol^{-1}cm^{-1}$at the shoulder (337 nm).

2.2 Steady-state fluorescence

Fluorescence emission behavior of DCM laser dye in a variety of solvents has been measured [11, 16]. DCM dye molecule exhibits a single structured fluorescence band in non-polar aprotic solvents. For example, the fluorescence maxima of DCM in isooctane and cyclohexane are found to be at 533 nm and 530 nm, respectively (Stoke shift of ~80 nm), which shifts its maxima (to 566 nm) upon increasing polarity of the solvent (1,4-dioxane). On the other hand, in dipolar aprotic and protic solvents, the fluorescence maxima shift towards the red region of the visible light, undergo a little change in shape of the band, and are accompanied by a new fluorescence band with its maximum above 610 nm. Similarly, the DCM emits at 635 and 626 nm in DMSO and DMF, respectively, that gives rise to ~150 nm Stokes shift. Furthermore, from the systematic fluorescence study, it was observed that the short-wavelength fluorescence intensities depend upon solvent polarity and that the intensity of the longer-wavelength band enhanced monotonically with increasing polarity of the solvent. The structured fluorescence emission band in non-polar solvent is attributed to the Franck-Condon or locally excited (LE) state where the DCM molecular structure/configuration is almost same as the ground-state configuration. The dynamic Stokes shift of the fluorescence emission maxima in polar solvents indicates that the nature of the emitting state is changing to a highly polar state and the solvation of DCM molecules further stabilizing the emitting state. From Stokes shift values obtained in different solvents and by using Lippert-Mataga theory, the excited-state dipole moment (μ_e) was estimated to be 26.3 D [16], which further supports the high dipolar nature of DCM emitting state. A large change in dipole moment (~20 D) from ground state to the excited state resulted in a large Stokes shift (~150 nm) from non-polar solvents to the polar solvents. The estimated μ_e and large change in dipole moment upon photoexcitation also explain why Stokes shift is more than the solvatochromic shift. Since μ_e is very high, it is likely that the DCM molecule mostly exists in the planar confirmation in charge-transfer (CT) state which will be relatively more stabilized by polar solvents rather than non-polar solvents. Further, it was observed that both the spectral shifts are correlating with Lippert-Mataga solvent parameter, Δf.

In order to understand the nature of the emitting state, titration experiments were carried out in which aliquots of pure ethanol solvent are added gradually to DCM and dioxane solution [9]. It was observed that the original fluorescence band in pure dioxane is redshifted upon gradual addition of ethanol to the DCM-dioxane solution and concurrently produces initially a longer-wavelength fluorescence band with a maximum at 610 nm which reduces its intensity beyond certain ethanol concentration (10^{-4} M) and emerged to a new fluorescence band with a maximum at 630 nm. However, further increase of ethanol concentration beyond this limit did not shift the position of the longer-wavelength fluorescence maximum but increases intensity of fluorescence band despite the fact that there is significant increase in the polarity of the binary solvent mixture. Fluorescence quantum yield (ϕ_f) of DCM highly depends upon the polarity of the solvent. For example, in n-hexane solvent, DCM quantum yield is calculated to be 0.05, and in polar DMSO solvent, quantum yield is estimated to be 0.81 [15]. Therefore, the quantum yield of DCM in non-polar solvents are less and in polar solvents high (**Table 1**). The observed high fluorescence quantum yields in polar solvents can be understood in terms of the CT character of the DCM dye. From the initial steady-state fluorescence studies, it was proposed that the DCM dye molecule emits a single fluorescence, and a three-state model was proposed in order to explain the fluorescence

spectral behavior, and all the solvents and the emitting state would be either LE state. Therefore, solvatochromic behavior of DCM was attributed to the change in their dipole moment of the ground state and excited state where fluorescence spectral shift increases due to an increased dipole moment upon excitation and to the interaction of this dipole with the polar solvent cage.

As can be understood from the molecular structure (**Figure 1**), the DCM dye can present in either cis-confirmation or trans-configuration because of π-spacer. So, the photophysics of cis- and trans-isomerization of DCM were studied by Drake and co-workers [10]. The DCM solutions were analyzed by high-pressure liquid chromatography (HPLC) and nuclear magnetic resonance (NMR), and they found that in the freshly prepared solutions, the DCM exists in trans-configuration (in dark). However, DCM solution when exposed to ambient light, trans-DCM converts in to cis-DCM whose ratio depends on the solvent. From HPLC study, absolute absorption cross sections for both isomers were measured for the first time. The fluorescence quantum yield of trans-isomer is found to be more than that of the cis-isomer because of the less non-radiative rate of the trans-DCM. Temperature dependence of the fluorescence emission spectra of both isomers in methanol, dimethylsulphoxide (DMSO), and lipid bilayers was studied [14]. These results suggest that the fluorescence spectral behavior of the two isomers is almost overlapping while their fluorescence decay times are found to be distinct. Furthermore, cis-DCM fluorescence was measured for the first time in DMSO solvent along with the trans-DCM, and it is observed that the cis-isomer fluorescence quenches to give the trans-DCM.

Based on the steady-state absorption and fluorescence studies in a variety of solvents, a mechanism has been proposed to understand photophysical properties (**Figure 2**) [9]. The DCM dye may be thought of an ionic merocyanine-like electron donoracceptor (EDA) dye molecule in which an electron-donating N,Ndimethylaniline moiety is covalently connected with a conjugated π-electron spacer and an electron-accepting dicyanomethylene moiety. Electronic excitation of DCM molecules leads to the formation of locally excited (LE) state immediately after photoexcitation. So, the fluorescence emission of DCM in non-polar solvents predominantly occurs from LE state, formed via π-π* transitions, and has an electronic configuration similar to that of the ground state, which is evident from the vibronic fluorescence emission band. However, in polar solvents, excited DCM molecules emitted from ICT state, which are characterized by a planar molecular conformation, are formed immediately after photoexcitation under the influence of the electric polarization of the surrounding solvent molecules, and it is argued that the short-wavelength fluorescence primarily originated from ICT state. This also explains why

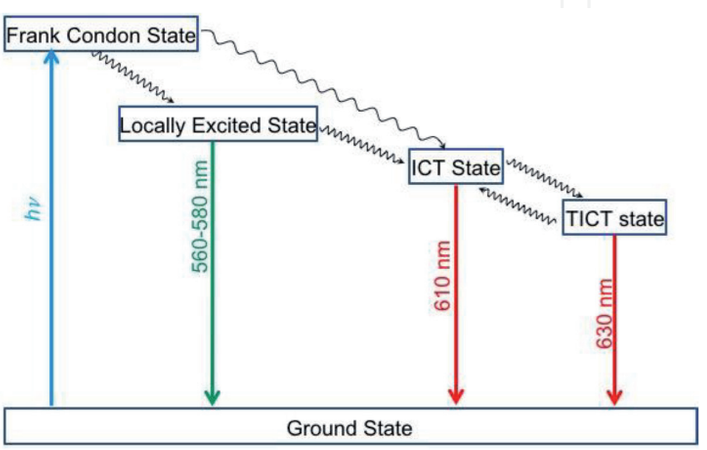

Figure 2.
Schematic diagram of the dynamic behavior of low-lying singlet states of DCM.

a gradual shift in the position of the fluorescence band is observed from a non-polar aprotic solvent to a polar solvent. Further, interpretation of the additional long-wavelength fluorescence was not that easy as expected; however, the preliminary fluorescence lifetime data suggest that it is generated from excited DCM in a new ICT state which is formed during the lifetime of the lowest excited singlet state and equilibrates with the ICT state emitting at 610 nm. It was suggested that the dual fluorescence originates from the excited DCM in the ICT state with a twisted conformation formed by internal rotation of the donor moiety with simultaneous ICT from this group to a suitable acceptor orbital. The new state is commonly known as twisted intramolecular charge transfer state (TICT) which was first reported by Grabowski and co-workers [21] to explain dual fluorescence of structurally different compounds such as p-cyano and p-(9-anthryl) derivatives of N,N-dimethylaniline in polar solvents [17, 22]. Typically, the TICT state is characterized by a perpendicular conformation of donor and acceptor moieties which is responsible for dual fluorescence of p-N,N-dimethylaminobenzonitrile (DMABN). However, unlike DMABN molecule, it should be noted that the difference between the short- and long-wavelength maxima of the dual fluorescence of DCM is somewhat smaller than that calculated for DMABN. This may be because the larger separation between the D and A moieties in DCM leads to a smaller fraction of charge transfer than that of DMABN.

Contrary to the above three-state model, a combined experimental and theoretical study revealed quite different results from the measured absorption and steady-state emission spectra of DCM dye upon its comparison with Nile red in a series of aprotic solvents with similar refractive index and different polarity [16]. Unlike many other studies reported earlier, the observed spectral behavior is interpreted to two-state electronic model accounting for the coupling to internal molecular vibrations and to an effective solvation coordinate. This study pointed out that change in band shapes upon varying solvent cannot be accounted as an evidence for two different emitting states and explained all the observed solvatochromic behavior of absorption and fluorescence spectra. Based on the consistency between experimental and calculated spectral data, a two-state model was suggested for understanding DCM photophysical properties which is generally also valid for most of the of the electron donor-acceptor (EDA) molecules.

2.3 Fluorescence lifetimes

Fluorescence lifetimes of DCM were measured in six different solvents for the first time, and it is found that the fluorescence times (τ) depend upon the polarity of the solvent [8]. Later on, wavelength dependent fluorescence decay profiles of DCM in protic-polar solvent (ethanol) and other solvents were measured, and it is found that all the decays profiles are fitting with single exponential function despite the strong overlap between the two fluorescence bands [9]. Moreover, these studies clearly reveal that the fluorescence lifetime value of DCM in a given solvent is independent of the fluorescence wavelength at which the measurement was made. In order to obtain more information about the nature of the emitting states of DCM in polar solvents, the fluorescence spectra of DCM in DMSO were recorded at various times after excitation. From typical time-resolved emission spectral data, it was observed that both short- and long-wavelength fluorescence bands appear within the 0.75 ns after excitation. Further, their relative intensities change with time until a time-independent intensity ratio is reached, at about 2.25 ns. Wavelength-dependent time-resolved fluorescence measurements also suggest that DCM exhibits dual fluorescence in polar solvents which is assigned to the two well-separated different emitting states. Based on the steady-state and time-resolved fluorescence data, Hsing-Kang and co-workers suggested two different intramolecular charge

transfer (ICT) emitting states for DCM which are in dynamic equilibrium with each other, where a short-wavelength emission was assigned to a planar conformation and a longer-wavelength emission to a twisted (TICT) conformation.

Fluorescence decay measurements of cis- and trans-isomers of DCM were carried out in six solvents using PRA photon counting system [10]. The fluorescence decays of DCM are fitting with mono-exponential despite the presence of two isomers. On the contrary, quite different results were observed when lifetime measurements are carried out using picosecond time-correlated single photon counting technique [23]. The fluorescent decay profiles in methanol, acetonitrile, and chloroform are fitting in bi-exponential. A short component (~25–48 ps) is having longer lifetime in methanol and acetonitrile solvents than that in chloroform. On the other hand, long component has a lifetime (τ) of 1.38 ns in methanol and chloroform solvents and τ ~1.94 ns in acetonitrile. Furthermore, fluorescence decay is fitting with single exponential function in DMSO with a lifetime ~2.25 ns. Thus, it is proved that solvent plays an important role in the non-radiative decay processes of the DCM in excited state which ultimately changes the fluorescence lifetimes. Bi-exponential nature of DCM clearly suggests the presence of two fluorescent species, and similarly, single exponential decay fitting in DMSO indicates single fluorescence species. The long-lived species are predominant in methanol, and acetonitrile solvent attributed to a trans-isomer which is produced while synthesizing DCM. From the relative weight component ratio ($a_2/a_1 + a_2$) analysis at a fixed excitation wavelength, it was observed that the relative contribution of the cis-DCM increases in the order methanol, acetonitrile, and chloroform which is inconsistent with the cis-DCM percentages obtained by Drake et al. [10] Further, a short component is attributed to cis-DCM with fluorescence lifetime in picoseconds and low fluorescence efficiency. The cis-DCM is having steric hindrance that inhibits planarity and rigidity of the molecule and thereby favors electronic to vibrational energy conversion. The decay behavior in highly polar DMSO solvent medium is attributed to relaxed fluorescence state from LE state, and it was pointed that TICT model is not necessary to describe single exponential decay [24]. Based on steady-state and time-resolved fluorescence studies, photoisomerization mechanism was suggested as follows: excitation of the trans-DCM followed by a nonadiabatic curve crossing process in which a surface crossing leads directly to the photoisomer. Another possible scheme would involve production of an intermediate, twisted internal charge transfer (TICT) state from the excited trans-configuration followed by partitioning to the cis and trans ground state which is similar to the Rullière model [25]. Solvatochromic absorption and emission behavior and the fact that the molecule possesses well-separated donor (amino) and acceptor (cyano) groups are consistent with the well-known charge-transfer properties. Therefore, it is likely that the geometrical configuration is skewed and intermediate between the cis and trans excited states. On the other hand, non-exponential fluorescence decay of DCM was observed at low temperature (5 and −35°C) in dibutyl ether, and the main fluorescent state was attributed to a TICT state [26].

2.4 Ultra-fast spectroscopic studies of DCM

As described in previous sections, since steady-state absorption and fluorescence studies were not conclusive about the nature of emitting state, one would always ask whether fluorescence emission is from direct charge-transfer state (CT) or relaxed CT state which is originated from locally excited state as shown in **Figure 3**. To answer this question, it is not necessary to have ultra-fast spectroscopy data; in fact simple steady-state fluorescence data would be sufficient to explain the nature of the emitting state. Suppose if it is encountered that the transition dipole moments for absorption (CT ← S_0) and emission (CT → S_0) are the same, one can conclude that the DCM photophysics are involved in two states (ground and CT states) [27]. Further, in such

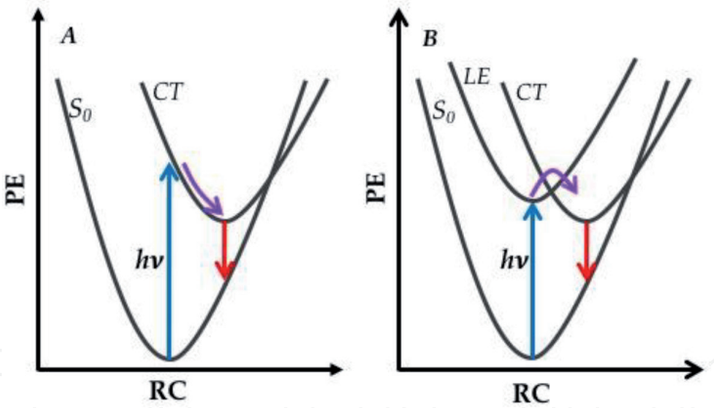

Figure 3.
Potential energy curves against generalized coordinates which include intramolecular and solvent modes for (A) direct vertical excitation to CT-state (B) population of LE state followed by LE → CT transition from ground state.

a case, the solvatochromism of absorption and emission should be consistent with ground- and excited-state dipole moments and their difference. On the other hand, if fluorescence anisotropy of DCM is substantially smaller than 0.4, then it is possible that the fluorescence emission could be from a different state than that of populated by photoexcitation, perhaps it is direct indicative of a three-state system (ground, LE, and CT states). Therefore, explicit evidence of such a three-state system can only be obtained by time-resolved spectroscopy through the direct observation of the LE → CT transition. However, because of the interference of both population transfer and relaxation (solvent, vibration) in spectral dynamics, the interpretation of the transient spectra can sometimes be sensitive and may to lead confusion. Easter et al. have investigated ultra-fast dynamics of DCM for the first time and observed temporal evolution of its stimulated emission in methanol and ethylene glycol at several wavelengths using sub-picosecond pump-probe spectroscopy [28]. The observed temporal changes of the fluorescence intensity measured during the first 100 ps after excitation were assigned to the dynamic Stokes shift of the fluorescence emission from the CT state following its direct optical excitation. Time-resolved transient absorption spectroscopic studies of DCM solutions in weakly polar and polar were carried out by Martin and co-workers, and corresponding data exhibits an isosbestic point in the net gain spectra within a few picoseconds after excitation which suggest rapid evolution of an emissive intermediate state from the initial excited S_1 state [29]. Solvatochromic behavior of the gain spectral position and its time-resolved redshift in slowly relaxing solvents support the CT character of the emissive intermediate state. Further, the overall intramolecular CT process is observed to take place within 30 ps in all solvents, and solvent relaxation time appears as an important parameter in the observed kinetics. Moreover, it was also found that the time constants associated with these changes depend upon the solvent polarity and vary from 2 ps (in acetonitrile) to 8 ps (in methanol). All these dynamics of DCM were interpreted to a transition that occurs from optically populated LE state to the CT state. However, there was no evidence of the twisted nature of this CT state which was suggested earlier [26].

Population relaxation within the fluorescent state was selectively monitored by Glasbeek and co-workers using femtosecond fluorescent up-conversion technique with a time-resolution of ~150 fs which does not permit to probe any influence of the dynamics within the electronic ground state [30]. It has been shown that intramolecular charge separation is taking more than 300 fs after the pulsed excitation. Following the pulsed excitation of the molecule, the integrated intensity of the spontaneous fluorescence decreased to approximately 50% of its initial value within few picoseconds. Moreover, it was observed that a significant portion of the charge

separation trajectory (~30%) is controlled by the solvation process on a picosecond time scale. Therefore, it is inferred that LE and CT states of photoexcited DCM strongly coupled adiabatically in the inverted region where a large extent of the charge separation process occurs on a picosecond time scale controlled by the excited state solvation process. However, subsequent high-resolution (<100 fs) fluorescence up-conversion studies of the DCM dye molecule in methanol and chloroform reveal that there is no change of the integrated spectral intensity during the first 25 ps after vertical excitation for the LE → CT transition [31]. Besides, for all times only one fluorescent excited state was noticeable, and the observed dynamic Stokes shift is attributed to solvent relaxation. Mean position of the time-resolved fluorescence spectrum of DCM in methanol shifts towards the red side with bi-exponential (175 fs and 3.2 ps) behavior, while in chloroform the spectral position remains practically unchanged for all times. The collected time-resolved data concluded that DCM has a single emitting state, which is directly populating upon photoexcitation.

A binodal dynamic Stokes shift was observed with time constants, one is about 100 fs, and another is of few picoseconds, respectively, when DCM is present in highly polar solvent media (methanol, ethylene glycol, ethyl acetate, and acetonitrile) [32]. The initial fast component is attributed to the free streaming motions of the solvent molecules and the second slow time component to the rotational diffusion motions of the solvent molecules. However, from the rapid sub-picosecond rise of the integrated emission intensity, it was suggested that the excited state electron transfer is preferentially taking place within about 100 fs from a higher-lying less emissive state to a lower-lying more emissive CT state. That is, the charge separation process in DCM is completed within about 100 fs. The LE and CT states are pictured as strongly coupled in the inverted region which is already reported earlier by Gustavsson et al. [31], and the gradual charge separation is treated as diffusional motion on the resulting barrierless potential. On the other hand, transient absorption spectra of DCM dye in methanol were measured using pump-supercontinuum probe technique with 40 fs time resolution and also revealed two components [33]. Initially (before 70 fs), a prominent spectral structure is observed which is primarily due to resonance Raman processes. At longer times (>70 fs), the spectrum undergoes a significant redshift, and shape of the band changes with a well-defined isosbestic point, and these observations are quite similar to earlier study done by Martin and co-workers [29]. The early transient component has been assigned to the locally excited state of DCM. Further, it was found that LE → CT transition is much faster than that suggested by Martin et al. and concluded that a substantial fraction of the intramolecular charge separation (≥70%) is completed within 300 fs of the pulsed excitation.

Later, time-resolved visible pump and infrared (IR) probe transient absorption measurements of the DCM and its isotopomer DCM-*d*6 were studied by Fleming and co-workers to probe the ultra-fast charge-transfer state formation in polar solvents: dimethylsulphoxide (DMSO) and acetonitrile (MeCN) [34]. Transient infrared absorption bands at both a fingerprint region between 1440 and 1620 cm^{-1} and the CN stretching region, ~2208 cm^{-1}, were probed. The IR band at 1440 cm^{-1} is assigned to the LE state of DCM, while the higher-frequency absorptions (1495, 1520, and 1590 cm^{-1}) are assigned to the CT state. The results reveal that excited-state absorption bands in the fingerprint region (1495 cm^{-1}) are exhibiting a frequency upshift and/or changes in band shape on a few ps time scale (~1–2 ps) which was attributed to the formation of the excited state and charge-transfer state via twisting and pyramidalization of the C–$N(Me)_2$ group and associated changes in C–C bonding character throughout the molecule. That is the fast rise in the CT bands was assigned to the rapid evolution of the LE state into the CT state.

Excited state non-radiative relaxation dynamics of DCM in hexane have been investigated using femtosecond fluorescence up-conversion technique at three

excitation wavelengths [35]. The S1 lifetime was observed to be 9.8 ps which is found to be independent of the excitation wavelengths. The observed S1 lifetime of DCM is less by one order of magnitude as compared to julolidyl DCM dyes DCJT and DCJTB, indicating the significance of the twisting motion of the N,N-dimethylamino group affecting the S1 non-radiative dynamics. Further, TDDFT calculations suggest that an intersystem crossing is responsible for the observed S1 dynamics of DCM in non-polar solvent.

2.5 What is understood about DCM dye?

The ground state and dipole moments of DCM are estimated to be very high (5.6 D and 26.6 D) which suggests that the charge is highly polarized even in the ground state. The steady-state absorption and fluorescence spectra of DCM reveal that the molecule exhibit solvatochromic shift and large Stokes shifts depending on the polarity of the solvent [10, 16, 24]. Solvatochromic shift of the electronic absorption is due to high ground-state dipole moment. The dramatic Stokes shift is attributed to the change of the dipole moment upon photoexcitation and fluorescent emitting state to a charge-transfer (CT) state [23, 24]. The fluorescence lifetime of DCM is measured to be of the order of a few nanoseconds, and the solvent relaxation occurs in between sub-picoseconds and picoseconds [9, 10, 23, 28–33]. Both fluorescence lifetime and relaxation depend on the solvent polarity.

Photoexcitation of DCM to its first absorption band put the excited molecule in the S_1/LE state, and subsequently two conformational changes may happen. Firstly, –C=C bond rotation leading to trans and cis isomerization via a phantom singlet state which is a typical photochemical process occurring on trans-stilbene [36] and many olefin molecules [37]. Secondly, twisting of the N,N-dimethylamino group may give rise to a highly polar twisted intramolecular charge-transfer (TICT) state which can be stabilized in polar media like 4-dimethyl-aminobenzonitrile (DMABN) molecule [37, 38]. However, the transition from the LE state to the CT (or TICT) state is under debate, and from both experimental and theoretical calculations [39], the following widely accepted dynamical behavior has been proposed to understand the excited-state dynamics of DCM dye. The potential energy surface (PES) of the LE state (S_1) for twisting motion of the central C=C bond (which bridges N,N-dimethylamino group with pyran group) is calculated to be very small (0.2 eV), and the barrier height is insensitive to the polarity of solvent. However, the shape of excited-state PESs of for the twisting motion of the CN single bond of the N,N-dimethylamino group of DCM is strongly influenced by the polarity of the solvent [39]. Moreover, in a polar media, the energy of the S_1/LE state increases, whereas the energy of the S_2/CT state decreases by twisting the CN single bond of the dimethylamino group and leads to a nonadiabatic curve crossing between the two states. Therefore, the formation of an emissive TICT state along the amino group twisting coordinate is more favored with increasing the polarity of the solvent. Trans and cis isomerization is dominated in polar solvents because of the increased the energy barrier in the TICT state along the torsional coordinate of the C=C double bond when the TICT state is formed at the perpendicular geometry where the energy of the S_1/LE state is higher than that of the S_2/CT state.

3. DCM derivatives as optical sensors

A *chemosensor* can be any organic or inorganic complex molecule that is used for sensing of an analyte to produce a detectable change or a signal [40–43]. Similarly, Cambridge defined the chemical sensor as a 'miniaturized device that can deliver

real time and online information on the presence of specific compounds or ions in even complex samples'. The chemical sensors employ specific transduction techniques to obtain analyte information. The chemical sensors are widely developed based on optical absorption, luminescence, and redox potential principles. Moreover, sensors based on other optical parameters, such as refractive index and reflectivity are also frequently reported in the literature [44].

Any chemosensor consists of three components: a *chemical receptor* which is capable of recognizing the analyte/guest of interest; a *transducer* or *signaling unit* that converts recognition event into a measureable physical change; and finally a method of measuring change and converting it to useful signal/information. An ideal chemosensor is expected to have high selectivity, sensitivity, prompt response, and low cost. Various approaches have been developed in the recent past years by various groups for designing chemosensors and broadly classified in to three different approaches [45], which only differ in the arrangement of receptor and signaling unit:

- Binding site-signaling approach
- Displacement approach
- Chemodosimeter approach

These approaches only differ in the arrangement of two units (receptor and signaling) with respect to each other. In the 'binding site-signalling subunit' approach, two parts are linked through a covalent bond. The interaction of the analyte/guest with the binding site induces changes in the electronic properties of the signaling subunit that results sensing of the target anion. The displacement approach is based on the formation of molecular assemblies of binding site-signaling subunit, which in coordination of a certain anion with the binding site results in the release of the signaling subunit into the solution with a concomitant change in their optical properties. In the chemodosimeter approach, a chemical reaction results in an optical signal when a specific anion approaches the receptor. Depending on the type of signals that are produced upon the recognition event, chemosensors are classified into two categories: optical sensors and electronic sensors. While the former sensors change optical signals, the latter change electrochemical properties. Based on the type of optical signal, the optical sensors further can be classified into two categories.

Chromogenic chemosensors change the color upon the recognition event (binding of analyte/guest into the receptor subunit) and thus show variation in absorption of signaling unit. Since the color of parent solution is changing after recognition, these are also known as colorimetric sensors.

Fluorogenic chemosensors change the fluorescence of the signaling unit upon the recognition event. These are also called fluorosensors.

It has been demonstrated that the colorimetric sensors are simple and low-cost and offer both qualitative and quantitative information without any need of sophisticated spectroscopic instrumentation, and most often the colorimetric response can be visualized with the naked eye. On the other hand, the fluorescence measurement is a bit expensive but relatively more sensitive and versatile and offers micro- to nanomolar estimation of guest species. A wide variety of optical chemosensors have been reported for the cation, anion, and neutral molecules. Based on the nature of analyte being detected, irrespective of the photophysical phenomenon the receptors follows, the chemosensors may be broadly classified into three categories: cations sensors, anions sensors, neutral sensors.

The ICT mechanism has been exploited quite extensively in ion sensing and molecular switching applications [45, 46]. A fluorosensor is generally designed to

have two units: a signaling unit typically a fluorophore and a receptor (recognition unit) which are covalently connected with a π-spacer for rendering the recognition event to the fluorophore that ultimately changes fluorescence signal. A group of fluorogenic sensors which has either weak fluorescence or no fluorescence (off state) by nature and that becomes fluorescent (on state) upon the receptor recognizes the analyte/guest molecule, and this type of fluorogenic sensors are called as off–on sensors. Similarly, on–off sensors can also be designed, where a sensor initially exhibits fluorescence (on state) and after the recognition event, the sensor becomes nonfluorescent/weakly fluorescent (off state). A schematic representation of off–on fluorogenic sensors is shown in **Figure 4**.

As discussed in the previous section, the DCM molecule and its derivatives are having unique advantages in terms of their photophysical properties such as red light emission, high quantum yield, and highly tunable fluorescence that is sensitive not only by solvent polarity but also structure modification. Unlike visible light fluorogenic sensors, red and NIR fluorogenic sensors (600–950 nm) have received considerable interest due to minimum fluorescence background, less light scattering, and less photodamage and are having certain advantages in bioimaging applications of live cells. Therefore, in recent years, there is a consistent growth of the colorimetric and fluorogenic sensors based on DCM and its analogues (**Figure 5**) for sensing cations, anions, and neutral species, which are summarized below.

3.1 DCM derivatives as metal sensors

Valeur and Bourson designed a DCM derivative, **DCM1**, which contains a receptor macrocycle (monoaza-15-crown-5) unit that is covalently attached to the electron-donating substituent (N,N-dimethylaniline unit) [47]. It was found that the resulting fluorosensor **DCM1** has almost identical photophysical properties to that of DCM. However, upon complexation with alkaline earth metal cations such as Li, Na, Mg, and Ca, the absorption spectra of **DCM1** undergo either hypsochromic shift or hypochromic shift. Similarly, with addition of alkaline metal cations, a substantial decrease in the fluorescence emission intensity and quantum yield was also observed. It is interesting to note that the fluorescence

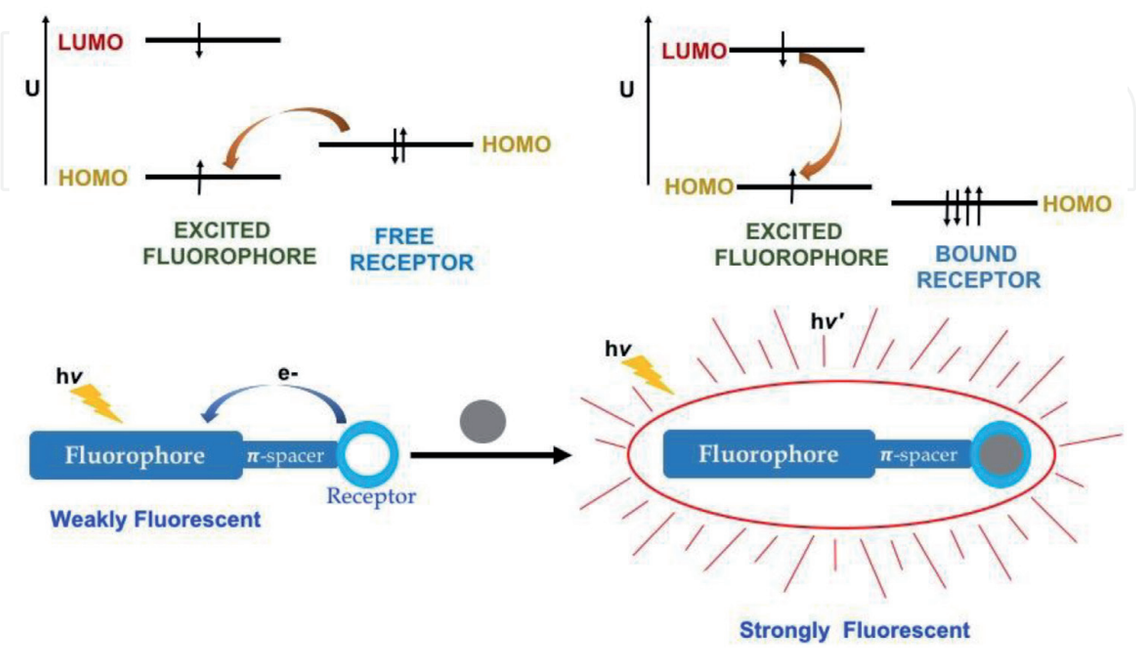

Figure 4.
Schematic diagram of OFF–ON fluorogenic sensing mechanism [45, 46].

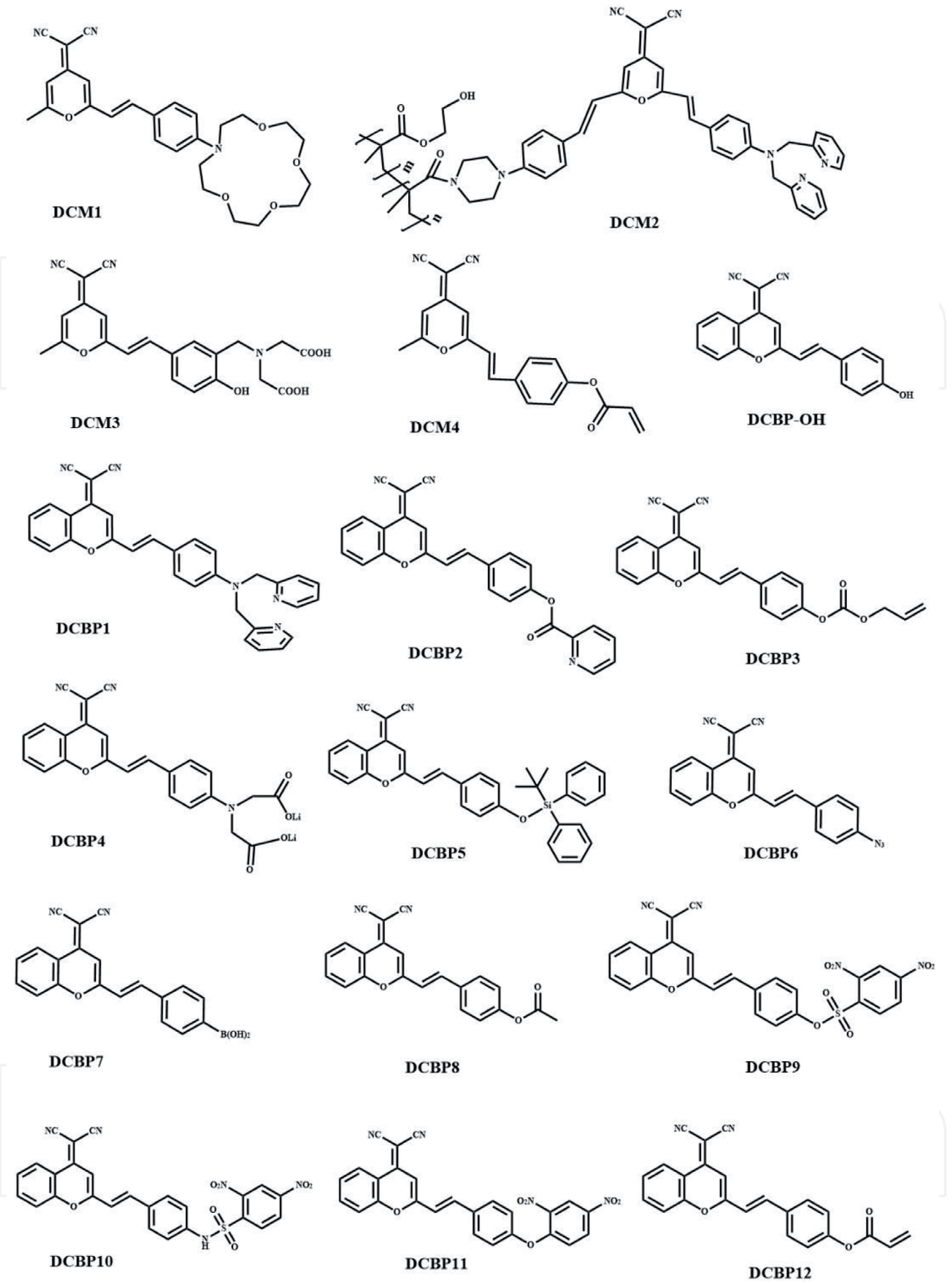

Figure 5.
Molecular structures of DCM and its derivatives as optical sensors for various analytes.

emission is slightly blueshifted and corresponding fluorescence lifetime is almost unchanged. It is well established that the ICT from the electron donor to the electron acceptor can be diminished if the electron-donating character of the donor moiety is reduced. In the **DCM1** fluorosensor, the nitrogen atom belongs to the crown, and therefore, upon complexation with cations, the donating ability is reduced and thus hinders the ICT character which more or less depends upon on the nature of the cation. From the observed sensing changes, it is understood that the charge transfer of the cation plays a key role and the reduction of

charge-transfer efficiency from a nonemissive locally excited state to an emissive relaxed intramolecular charge-transfer state (RICT).

A red fluorosensor (**DCBP1**) was designed by replacing N,N-dimethylamine of **DCM** with bis(2-pyridylmethyl) amine (DPA) moiety and benzopyran backbone [48]. Screening of various alkaline and transition metals reveals that the **DCBP1** has more binding affinity towards Cu^{2+} ions than that of any other cations. The binding affinity is evident not only from the absorption spectrum but also colorimetric response where light pink color of free **DCBP1** solution changes to yellow color after coordinating with copper ions (**DCBP1-Cu2+**), which is visible even to the naked eye. Free **DCBP1** shows a characteristic emission band around 650 nm (with fluorescence high quantum efficiency, ϕ_{DCBP1} = 0.40) which is redshifted 55 nm as compared to fluorescence emission of **DCM** (λ_{em} = 595 nm) due to insertion of a conjugated benzene unit onto the dicyanopyran backbone. Fluorescence behavior of **DCBP1** in presence of various metal ions was studied in a mixture of ethanol-water (60:40, v/v), and it is observed that only the addition of Cu^{2+} to **DCBP1** causes a significant decrease in fluorescence intensity. Surprisingly, when pyrophosphate (PPi) anion is added to the in situ generated **DCBP1** meatal complex **(DCBP1-Cu2+)**, the absorption at 505 nm increases with a isosbestic point at 447 nm, and the color of the solution also changes from pale yellow to pink (original color of **DCBP1**). Similarly, fluorescence emission of the **DCBP1-Cu2+** is turned on, and fluorescence emission intensity at 650 nm is also enhanced. From the fluorescence measurements, it is observed that **DCBP1** forms a 1:1 complex with pyrophosphate (PPi) anion, and association constant (K_a) is estimated to be very high at 4.6×10^5 M. Further, an investigation of a series of other anions reveals that the **DCBP1** probe molecule is highly selective and sensitive only towards PPi anion. The observed colorimetric response and on–off fluorescence response of DCBP1 were attributed to the inhibition of the ICT because of decreased the electron-donating ability of the amino group upon binding with Cu^{2+} ion. On the other hand, turn-on fluorescence is due to electrostatic interaction between PPi and **DCBP1-Cu2+**. Since, the two oxygen atoms of PPi somewhat strongly coordinated with the copper, and the nitrogen-copper bond gets weakened which restores the ICT; thereby fluorescence emission is enhanced. Therefore, the **DCBP1** molecule is demonstrated as both fluorescence on–off and off–on sensor when it is binding with Cu^{2+} ions and PPi, respectively.

In general, most of the fluorosensors exhibit on–off sensing behavior in solution phase because quenching of fluorescence emission is quite easy. However, developing off–on fluorosensor with processible technology is relatively a tedious and challenging task. Such fluorescence off–on sensors can be tailored to meet the specific needs via rational design approaches and have been paid much attention in recent years due to growing demand of various chemical and biological species detection by exploiting energy transduction principles such as radiant, electrical, mechanical, and thermal processes [49, 50]. Tian and co-workers have extended their previous research work [48] and developed a polymeric **DCM2** sensor based on a hydrophilic copolymer bearing the **DCM** moiety in the form of a fluorescent film which senses Cu^{2+} and PPi anion works based on off–on fluorescence mechanism (**Figure 6**) [51]. The sensor **DCM2** is decorated with a hydrophilic copolymer, poly(2-hydroxyethyl methacrylate) (PHEMA), that exhibits high hydrophilicity but insoluble in water. The hydrophilic chain segment was chosen mainly to improve the permeability of ions into the polymer backbone, and the **DCM** fluorophore is also grafted into the polymer backbone as metal ion-sensing units. The copolymer **DCM2** and the corresponding metal complex, **DCM2–Cu2+**, exhibit turn-off fluorescence for the selective targeting of Cu^{2+} (**Figure 6**). However, interestingly, upon adding PPi anion, the fluorescence of the copolymer is turned on with high sensitivity both in solution and in thin film over other anions such as AMP, ADP, ATP, and phosphate (Pi). Furthermore, the low-cost

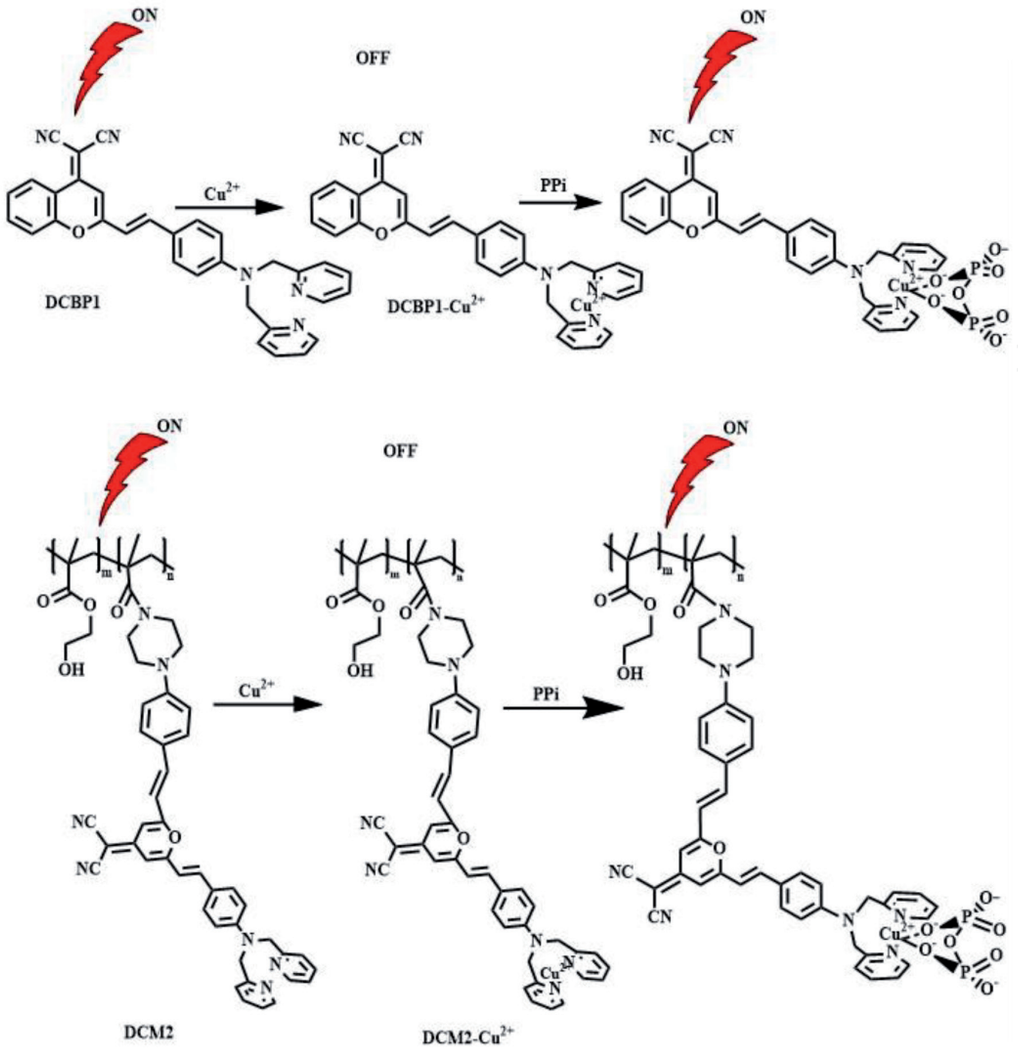

Figure 6.
Fluorescence on–off and off–on mechanism of DCBP1 (above) ***DCM2*** *copolymer (below).*

hydrophilic copolymer film of **DCM2–Cu2+** on a quartz plate shows a very rapid response towards PPi anion with turn-on orange-red fluorescence due to high permeability of its side chains. Recently, a new **DCM**-based NIR fluorescent probe (E)-4-(2-(4-(dicyanomethylene)-4H-chromen-2-yl)vinyl) phenyl picolinate (**DCBP2**) was designed and synthesized for Cu^{2+} ions with improved performance [52]. As shown in **Chart 5**, the sensor molecule **DCBP2** consists of **DCBP-OH** as a fluorophore and electron-withdrawing 2-pyridinecarbonyl group as the receptor for Cu^{2+} ions. The reaction between **DCM-OH** and 2-pyridinecarbonyl gives rise to picolinoyl ester of the **DCM**. Since 2-pyridinecarbonyl group is covalently anchored to the fluorophore, the ICT is blocked, and no fluorescence can be observed. At this stage when Cu^{2+} is added to the probe, the copper ions coordinate with nitrogen and oxygen atoms of 2-pyridinecarbonyl group fluorescence emission quenches. However, upon hydrolysing **DCBP2** with water, Cu^{2+} releases from coordination, and subsequently a phenolate ion (**DCBPO−**) is produced, which is a better electron-donating group and thus restores its ICT property which ultimately leads to a dramatic increase in fluorescence intensity at 676 nm. The sensing behavior of the probe **DCBP2** towards Cu^{2+} ions can also be conveniently followed by naked eye inspection and measuring absorption under mild conditions. The color of the solution appears yellow in absence of copper ions, which, however, changes to pink color upon adding copper ions and clearly visible to the naked eye. The free **DCBP2** gives absorption 558 nm upon, and binding with copper ions, the absorption shifts 415 nm (blueshift). Furthermore, this probe was

successfully applied for the quantitative estimation of Cu^{2+} in various types of water samples and also demonstrated its utility in imaging living cells.

DCBP3 is designed based on the Pd(0)-catalyzed Tsuji-Trost allylic oxidative insertion reaction and dicyanomethylene benzopyran moiety [53]. Photophysical properties revealed that the probe **DCBP3** exhibits high sensitivity and selectivity towards the detection of both Pd(0) and Pd(II) under reducing conditions. The probe **DCBP3** shows a major absorption band with a maximum at 450 nm, and after treating with palladium, another new absorption peak started appearing around 560 nm. On the other hand, **DCBP3** displays no fluorescence at 700 nm when excited at 560 nm. However, upon the addition of palladium, the fluorescence emission peak at 700 nm increases gradually. Additionally, marked color changes were also noticed. All the photophysical properties have been explained based on palladium-triggered cleavage reaction that produced a free **DCBP-OH**. Moreover, the probe **DCBP3** is little affected with pH variation and has low cytotoxicity.

3.2 DCM derivatives as anion sensors

As discussed in Section 3.1, the molecules **DCBP1** and **DCM2** form copper complexes (**DCBP1 Cu2+** and **DCBP1-Cu2+**), and their fluorescence emission quenches drastically [48, 51]. In situ generated **DCBP1 Cu2+** and **DCBP1-Cu2+** recognize PPi anion which can be tracked from spectrophotometrically and fluorescence measurements. Later, the molecule **DCBP1** was modified by decorating with a lithium iminodiacetate group in place of N-aryl group [54]. The synthesized NIR fluorophore, **DCBP4**, selectively binds with Cu^{2+} ions because of lithium iminodiacetate receptor and found to have very good solubility in aqueous water. The photophysical properties of metallated fluorophore (**DCBP4-Cu2+**) were found to be modified upon interacting selectively with pyrophosphate (PPi) anion. When PPi is gradually added to the solution of **DCBP4-Cu2+**, a new redshifted peak at 503 nm appeared and increased gradually with an isosbestic point at 450 nm. The absorption spectral changes are very much evident to the naked eye where the pale brown color of the **DCBP4-Cu2+** solution changes to red color. On the other hand, simultaneously turned on fluorescence and emission intensity in the NIR region (675 nm) are enhanced gradually and stabilized upon the addition of 15 equiv. of PPi. The fluorescence off–on switching and the colorimetric response of **DCBP4-Cu2+** are interpreted in terms of ICT variations upon sensing the receptor.

A near-infrared (NIR) fluorescent chemosensor, **DCBP5**, was developed on the basis of dicyanomethylene-4H-benzopyran derivative for detecting fluoride anions [55]. Chemodosimeter **DCBP5** was synthesized by the Knoevenagel condensation of 4-dicyanomethylene-2-methyl-4H-pyran and 4-(tert-butyldiphenylsilyloxy) benzaldehyde. With the addition of F^- ions to the **DCBP5** sensor, absorption band cantered at 447 nm slowly decreases, and at lower F^- concentration (<30 μM), a new absorption emerges at 454 nm gradually. When the F^- concentration was further increased beyond 50 μM, the new absorption band at 454 nm decreases, and a concomitant increase of a new band at 645 nm was observed with an isosbestic point at 510 nm. The large redshift (190 nm) is also noticeable to the naked eye in which the initial pale yellow color of the **DCBP5** solution changes to blue color upon adding fluoride ions. It should also be noted that the sensing process is very fast, and within 30 s the sensing is noticeable to the naked eye. The observed isosbestic point of **DCBP5** sensor upon addition of the F^- ions clearly indicates formation of a new species which is attributed to phenolate group generation due to Si–O cleavage. Similar supporting results were also observed from fluorescence measurements. The **DCBP5** molecule is non-fluorescent due to the presence of silyl group. However, the sensor **DCBP5** turn-on fluorescence with gradual addition of

F^- ion which is evident from the fluorescence emission measurements in which a new fluorescent band started emerging in the NIR region (at 718 nm). Since the in situ generated phenolate group is a much stronger electron-donating group than the silyl group, the ICT efficiency restored after **DCBP5** interaction with that of F^- ions. Further, the results also revealed that the **DCBP5** is not just an off–on fluorescent sensor, but it is also a ratiometric and colorimetric sensor which is the ideal characteristic of any sensor.

3.3 DCM derivatives for detection of neutral species

3.3.1 Hydrogen sulphide (H_2S)

Hydrogen sulphide (H_2S) is involved as a signaling molecule in various physiological processes that include modulation of neuronal transmission, regulation of release of insulin, relaxation of the smooth muscle, and reduction of the metabolic rate [56, 57]. From the animal model study of critical illness, it was realized that the H_2S donor protect from lethal hypoxia and reperfusion injury and exert anti-inflammatory effects [58]. Physiological H_2S concentration is estimated to vary from nano- to millimolar levels [59], and once this limit is crossed, the cells release H_2S that can cause certain diseases, such as Alzheimer, Down syndrome, diabetes, and other diseases of mental deficiency [60]. Hence, a reliable in vivo study is essential to measure accurately H_2S concentration thereby preventing deceases. A NIR probe, **DCBP6**, that comprises dicyanobenzopyran and 4-azidostyryl group as receptor was developed for selective detection of H_2S [61]. The probe **DCBP6** selectively reacts with H_2S and reduces the azido group ($–N_3$) to amine ($–NH_2$), and the corresponding molecule becomes highly fluorescent than the parent **DCBP6**. Upon H_2S detection, the **DCBP6** probe solution changes which is visible to the naked eye and causes a large Stokes shift (>100 nm in different solvents). Besides, the reduced probe **DCBP6NH2** exhibit two-photon absorption (TPA) which is having more advantages than traditional one-photon absorption probes in fluorescence microscopy such as less phototoxicity, better three-dimensional spatial localization, deeper penetration depth, and lower self-absorption. Further, the probe molecule **DCBP6** was successfully used as fluorescent probe for monitoring H_2S in living cells and tissues and in vivo in mice via fluorescence bioimaging investigations. More or less at the same time, Xu and co-workers have reported the same molecular probe for in vivo detection of H_2S [62].

3.3.2 Dopamine

A catecholamine compound dopamine is known as a neurotransmitter that regulates a wide range of cognitive functions such as behavior, learning, motivation, and memory [63–65]. The dopamine content in the human brain is an important factor that can cause various diseases that include Parkinson's disease, and in fact it is used as a marker in the diagnosis of several conditions related to neurotransmitters. Therefore, there is a strong quest for developing efficient and rapid methods that can selectively determine and continuously sense the dopamine levels on a real-time basis. The DCM fluorosensor (**DCM3-Fe2+**) was developed for selective detection of dopamine based on on–off sensing mechanism [66]. The electron-donor part of DCM fluorophore is modified with a ligand, diethyliminodiacetic acid, such that it selectively complexes with iron(II) ions. In the absence of dopamine, the sensor molecule **DCM3-Fe2+ is** weakly fluorescent due to inhibition of ICT because of Fe^{2+} complexation with the donor moiety (off-state fluorescence). However, a much stronger fluorescence emission was observed upon gradual addition of dopamine owing to the release of Fe^{2+} from DCM complex. A good linear relationship was

observed between the dopamine concentration and the fluorescence intensity. That means the observed fluorescence enhancement which is observed after addition of dopamine serves as an indicator to monitor dopamine content in a given sample. Besides, the fluorosensor does show any fluorescence response against other foreign substances, thereby allowing selective detection of dopamine.

3.3.3 Hydrogen peroxide (H_2O_2)

Zhang et al. have synthesized a new NIR and colorimetric fluorescent molecular probe, **DCBP7**, by covalently attaching dicyanomethylene-4*H*-benzopyran and phenylboronic acid for rapid detection of H_2O_2 [67]. The boronic acid functional group is attached primarily to have NIR fluorescence off–on switching. The sensing of H_2O_2 was successfully demonstrated by UV–visible absorption and fluorescence measurements. **DCBP7** exhibit a structured absorption band at 450 nm, and its solution appears pale yellow in color. However, with gradual addition of H_2O_2 (>20 equiv.), apparently, the absorption at 450 nm decreases, and a new absorption band starts evolving at 560 nm. Due to the large redshift (110 nm) of the absorption, the color of the solution (yellow) changed to purple, and colorimetric detection of H_2O_2 is visible even to the naked eye. The fluorescence measurements were also carried out to confirm the H_2O_2 sensing behavior of the probe molecule. The free probe molecule is non-fluorescent primarily because of phenylboronic group. However, after adding H_2O_2, the boronic acid group gets cleaved and generate a phenolate ion which is evident from the new fluorescent emission band at 670 nm. The Stokes shift (110 nm) of the phenolate band in the NIR region has been exploited further for detecting H_2O_2 and imaging live cells. Unlike the most conventional fluorescent probes, the developed **DCBP7** has been shown to have unique advantages such as deeper tissue penetration ability, lower background autofluorescence, and less damage to biological samples which ultimately allowed to in vivo studies of live cells.

3.3.4 Hydrazine (N_2H_4)

Hydrazine is used as a common precursor in synthetic chemistry of many polymers, pharmaceutical intermediates, hydrazine fuel cells in power generation sector, and materials science [68, 69]. It is often used in rocket propulsion systems as an important propellant for its flammable and detonable characteristics. Moreover, hydrazine serves as an important metal corrosion inhibitor because of its strong reducing properties; hydrazine scavenges oxygen in water boilers that are used for feed and heating systems. However, hydrazine and its aqueous solutions are highly toxic to all living organisms when inhaled or in contact. It has been shown that hydrazine is mutagenic and carcinogenic which causes serious damage to the human central nervous system, kidneys, liver, and lungs [70]. Therefore, it is of great interest and importance to develop a reliable method for hydrazine detection with selectivity and sensitivity. With a view to develop efficient DCM-based NIR fluorophore for selective detection of hydrazine, a phenyl ring baring *O*-acetyl moiety was introduced onto the into dicyanomethylene-4*H*-benzopyran backbone and synthesized DCBP8 [71]. The absorption and fluorescence properties of DCBP8 were measured in PBS solution (pH = 7.4) containing 50% of ethanol. DCBP8 has absorption in between 300 and 450 nm region with a maximum at 434 nm. After treatment of DCBP8 with N_2H_4, gradually new absorption peaks started appearing at 551 nm at the expense of 434 nm absorption band. The absorption maximum shifted from 434 nm to 551 nm which indicates the efficiency of DCBP8 for colorimetric detection of N_2H_4 when absorption intensity ratio (A_{551}/A_{434}) and concentration of N_2H_4 ranging from 0 to 40 μM are plotted against each other, there is a

good linearity suggesting a ratiometric response. On the other hand, free DCBP8 is almost no fluorescent upon excitation at 560 nm, which showed a dramatic fluorescence enhancement at 680 nm upon addition of N_2H_4. The fluorescence enhancement is found to be more than 110-fold. Mass spectrometry data along with photophysical properties revealed that the N_2H_4-medicated acetyl deprotection of DCBP8 generates **DCBPO−**, a highly fluorescent product because of ICT character.

3.4 DCM derivatives for sensing biothiols and selecysteine

3.4.1 Biothiols

There is quest for developing molecular probes for rapid, selective, and sensitive detection of the highly toxic thiophenols which are of great importance in both environmental and biological science. James and co-workers have developed a novel near-infrared (NIR) and colorimetric fluorescent molecular probe, **DCBP9**, based on a dicyanomethylene-4H-pyran chromophore for selective detection of glutathione in living cells [72]. The molecular probe **DCBP9** was synthesized by Michael's addition of 2-(2-(4-hydroxystyryl)-4H- chromen-4-ylidene) malononitrile and 2,4-dinitrobenzene-1-sulphonyl chloride (DNBS) in the presence of pyridine at room temperature. Molecular probe has an intense absorption centred at 414 nm in a DMSO-PBS buffer solution; upon the addition of glutathione (GSH), the color of the solution turned to pink from slight yellow and clearly visible to the naked eyes. In addition, a new absorption band emerged at 560 nm with an isosbestic point at 446 nm which is assigned to the specific O–S cleavage, and the generation of phenolate ion with a distinct 146 nm redshift in absorbance is observed. Since the phenolate group is a much stronger electron donor than the sulphonate group, the ICT efficiency is significantly enhanced by the interaction of **DCBP9** with GSH and thus shifts the absorption to a longer wavelength region. Subsequent fluorescence experiments showed that the molecular probe alone is nonemissive (turn-off) in absence of GSH. However, when the probe is excited at 560 nm in presence of GSH, turn-on fluorescence and the intensity at 690 nm were dramatically enhanced. The turn-on fluorescence is due to the release of electron-withdrawing DNBS moiety via a GSH-induced O–S bond cleavage and produces phenolate ion, which possesses a strong ICT character and induces a turn-on NIR fluorescence. Having known chemical properties of thiophenols that are able to cleave sulphonamide selectively and efficiently under mild conditions, a dicyanomethylene-benzopyran-based NIR fluorescent probe **DCBP10** is designed for detection of thiophenols [73]. Upon adding thiophenols to the **DCBP10** solution, the DNBS moiety is cleaved and forms amine ($–NH_2$) functional group at the phenyl ring. Since the amine is an electron-donating group, the ICT of the fluorophore is restored, and as a result, absorption and fluorescence emission properties of the probe were changed. This probe features remarkable large Stokes shift and shows a rapid, highly selective, and sensitive detection process for thiophenols with significant NIR turn-on fluorescent response. Therefore, **DCBP10** was successfully demonstrated as a potential NIR fluorescent probe that can be mitigated not only for quantitative detection of thiophenol in real water samples but also fluorescence imaging of thiophenol in living cells [73].

Slightly similar molecular structure **DCBP11** is redesigned by Li et al. that consists of dicyanomethylene-benzopyran scaffold and 2,4-dinitrophenyl (DNP) connected by ether linkage for probing for thiophenols [74]. It was demonstrated that **DCBP11** shows both colorimetric and rapid turn-on fluorescence sensing process for thiophenols with high selectivity and better sensitivity (DL = 70 nm). Moreover, it should be noted that a dual colorimetric and selective NIR fluorescence sensing phenomenon is also visual to the 'naked eye' without the need of advanced

instrumentation. In addition, quantitative detection of thiophenol in real water samples and fluorescent imaging of thiophenol in living cells and zebrafish were successfully demonstrated which suggests that this probe has a great potential for in vitro and in vivo applications. Another NIR probe, which contains a conjugated dicyanomethylene-benzopyran moiety as the NIR fluorophore **DCBP12** and an acrylate moiety as a receptor, is found to be promising for biothiols: cysteine (Cys), homocysteine (Hcy), and glutathione (GSH) detection. **DCBP12** itself almost non-fluorescent due to alkene-induced quenching of photoinduced electron-transfer (PET) process; however, it becomes fluorescent upon sensing biothiols. It has been proven that the NIR fluorescence enhancement of **DCBP12** is due to Cys sensing originating from cleavage of acryloyl group of **DCBP12** that simultaneously releases a NIR fluorescent DCM-OH. A feasible sensing mechanism was proposed to understand the sensing process of Cys. The reaction of Cys with **DCBP12** involves two steps. Initially, a Michael addition reaction of the thiol functional group of Cys with the acryloyl group has taken place and then followed by a spontaneous intramolecular cyclization to release the NIR fluorescent, phenolate ion (**DCMO−**). Further, the detection limit for Cys was estimated to be 81 nm. In addition, imaging of biothiols by **DCBP12** was also successfully demonstrated in living cells which indicates that this probe is suitable for imaging biological samples [75]. Therefore, **DCBP12** shows rapid response and high-selectivity and high-sensitivity biothiols particularly for Cys and Hcy, accompanied by distinct color changes seen by the naked eye and significant NIR turn-on fluorescence responses.

Recently, a red-emitting fluorescent probe **DCM4** was developed for selective detection of cysteine (Cys) over glutathione (GSH) and homocysteine (Hcy) by incorporating acryloyl group as the recognition unit into the 2-(2-(4-hydroxystyryl)-6-methyl-4H-pyran-4-ylidene) malononitrile (P-OH) fluorophore [76]. Selective detection of Cys is very important because, among biothiols, Cys is considered as the most significant biothiols of living organisms and plays a crucial role in multiple physiological processes that include mitochondrial protein turnover, protein biosynthesis, detoxification administration, and metabolism regulation [77]. Further, because of crucial physiological and pathological significance of Cys in biological systems, it is essential to develop a rapid and promising analytical tool for selective detection of Cys so as to unravel hidden physiological processes of Cys and understand the specific pathogenesis of Cys-related diseases. Basically, the probe design is almost similar with **DCBP12** used for thiol detection and **DCBP3** used for palladium detection. The **DCM4** molecule is almost non-fluorescent due to acryloyl group that blocks ICT and promotes non-radiative processes. Upon the addition of Cys, **DCM4** undergoes Michael addition of Cys and the acryloyl group to afford a transient intermediate, followed by the intramolecular cyclization to give highly fluorescent oxide anion. Therefore, accordingly by monitoring fluorescence intensity variations before and after the addition, the Cys can be detected. The fluorosensor **DCM4** has certain advantages. Firstly, probe **DCM4** has good selectivity for Cys over Hcy and GSH. Secondly, the probe senses Cys in the solution and responds in a short time (4 min) towards Cys. Thirdly, this probe exhibits high signal-to-noise ratio (~147-fold) and ultralow detection limit (41.696 nm). Thus, the **DCM4** was successfully demonstrated as off–on fluorosensor to monitor the Cys level in living cells with low cytotoxicity.

3.4.2 Selenocysteine

Selenocysteine (Sec) is a cysteine (Cys) analogue which consists of selenol group in place of the thiol group in Cys and considered as a major form of biological selenium and known as the 21st proteinogenic amino acid that is specifically

incorporated into selenoproteins (SePs). More than 50 human proteins are known to contain Sec [78]. Therefore, detection of Sec in physiological conditions is very important. In order to achieve NIR turn-on fluorescent detection of Sec selectively, the molecule **DCBP11** was designed which was originally used for thiol detection [74]. **DCBP11** senses the presence of Sec and shows colorimetric and NIR turn-on fluorescence response upon cleavage of ether bond and subsequent formation of **DCM-OH** [79]. Similar to many other DCM-based ICT molecules, **DCBP11** also shows a remarkable large Stokes shift at 146 nm. Besides, **DCBP11** is highly sensitive to Sec and exhibits a very small detection limit of 62 nm over a wide linear range (0.2–80 μM) of selenocysteines which allows quantitative estimation of the Sec. Moreover, it was further demonstrated that this NIR fluorescent probe can be employed to image both exogenous and endogenous Sec in living cells, indicating that **DCBP11** has great potential for biological applications.

3.5 DCM derivatives as pH sensor

A pH-sensitive fluorescent chemosensor, **DCBP-OH**, was designed based on dicyanomethylene-4H-benzopyran scaffold by employing D-π-A architecture [80]. At neutral pH, the **DCBP-OH** shows absorption at 450 nm which is attributed to the typical ICT band of DCM chromophore and very weak fluorescent (~ 574 nm). Interestingly, as the pH of the solution increases (from 7.15 to 11.00), the weak fluorescence emission band at 574 nm decreases, and simultaneously a new band at 692 nm started increasing. The evolution of new fluorescence band at 692 nm is assigned to the increase of the ICT process from the oxygen anion of phenolate group. That means the strong change in fluorescence intensity is a clear indication for determining pH of any solution from 7 to 11. Acid dissociation constant pK_a value is calculated to be 7.21. Moreover, it was found that the fluorescence signal ratio (I_{692}/I_{574}) is found to be ratiometric induced by a large Stokes shift of about 118 nm. Furthermore, from the absorption and fluorescence measurements, it was proved that the pH response of **DCBP-OH** is reversible which makes **DCBP-OH** a simple naked-eye sensitive NIR fluorescent chemosensor for pH measurement. In a very recent work, the **DCBP-OH** probe has been slightly modified with triphenylphosphate and shown as NIR sensor for lysozyme detection in urine sample [81].

3.6 DCM derivatives as polarity sensor

Kwak et al. developed different types of copolymers by decorating with the DCM moiety into a certain polymer chain which are sensible to external environment and useful to probe dye molecules [82]. The photophysical properties in solution, solid film, and aggregation revealed that ICT characteristics of the copolymers are modifying. More interestingly, it was observed that the fluorescent properties of DCM-type dyes within the polymers are significantly dependent upon the polarity of the polymer matrix. Three copolymers (P(St-*co*-**2**), P(MMA-*co*-**2**), and P(AN-*co*-**2**)) have shown quite unusual photophysical properties which are completely different from the corresponding DCM-type monomer. The copolymers show a blueshift in fluorescence emission relative to the monomer. The aggregates of copolymers prepared in polar medium (DMF) by adding methanol showed a significant blueshift in fluorescence emission, and aggregates prepared from non-polar medium (1,4-dioxan)/methanol exhibit a prominent redshift. Similarly, it was also observed that the fluorescence intensity of P(St-*co*-**2**) and P(MMA-*co*-**2**) decreased by aggregation while that of P(AN-*co*-**2**) increased. Such interesting solvatochromism and unusual aggregation behavior of the three copolymers were exploited further for selective sensing of volatile organic compounds (VOC).

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