Photophysical modulations of biologically potent small molecules in biocompatible microheterogeneous environments created by cyclodextrins and lipid vesicles

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Small molecules having biological potency are applied in all the avenues of chemical, pharmaceutical, and biological research. Typically, the small molecules are delivered to the biological environment using a compatible host that acts as a carrier for the compound. The internal environments of the hosts are typically non-polar or less polar than the bulk aqueous environment so that the potent drugs, most of them being hydrophobic, can get encapsulated to be carried to the target. On incorporation, the photophysics of the compounds may change as compared to the external atmosphere. This may alter their inherent properties and mode of functioning. In this perspective article, the relevant progress in host-guest chemistry in biologically potent environment aided by cyclodextrins and lipid vesicles has been discussed.

Keywords: Small molecules, photophysics, cyclodextrins, lipid vesicles, microheterogeneity, environment

Electromagnetic radiation can interact with a molecule in many different ways that can absorb and scatter the light. The absorption of radiation of different energies by a molecule gives vivid information about its energy levels, electronic distribution, structure, etc. Photophysical and photochemical processes in a molecule are initiated by the absorption of electromagnetic radiations of suitable wavelengths in the ultraviolet and visible regions. The excited molecule eventually comes back to the ground state by dissipating the excess acquired energy either through radiative or non-radiative pathways. Since the electric vector of the incident light is responsible for the creation of a dipole moment during the act of absorption, a difference in the strength of absorption is expected for different orientations of the anisotropic molecule. Photophysics of the guest molecules changes profusely when included inside hosts such as cyclodextrins (CDs), micelles, lipids, nucleosides, etc.

Cyclodextrin inclusion complexes

Because of the relatively hydrophobic cavity in comparison to the hydrophilic exterior, CDs can form inclusion complexes with hydrophobic guest molecules in aqueous solution predominantly due to hydrophobic interactions. However, the possibility for guest inclusion due to hydrophobic interactions is only one of the requirements, the other being the matching size of the guest molecule and the CD cavity. It must be emphasized that the phenomenon of CD inclusion complex formation is a complicated process and in reality, there are many factors that play their role. This is illustrated by the fact that not only apolar compounds can be included in CDs but also acids, amides, small ions and even rare gases\textsuperscript{1-3}. The 1:1 stoichiometry is most common among small molecule-CD complexes. Complexes of other stoichiometries such as 1:2, 2:1, 1:3, 3:1, 2:2, 1:1:1, and 1:1:2 are also frequently observed\textsuperscript{4-7}. Even for a given guest–CD pair, complexes of different stoichiometries can form. Examples of this are naphthalene\textsuperscript{8} and pyrene\textsuperscript{9} complexes of CD, which were studied extensively by fluorescence techniques. It is also possible that the guest is only partly included in one CD. Encapsulation of a polarity sensitive fluorophore inside the CD cavity produces a remarkable change in its fluorescence intensity and wavelength of maximum emission\textsuperscript{9-15}. The guest-host complex formation is a complicated phenomenon and depends on many factors. The hypsochromic shift of emission maximum on CD encapsulation is caused by the lower polarity of the CD cavity. Under such circumstance, the first singlet excited state of the fluorophore gets destabilized resulting into a blue
shift of the emission band. CD cavities provide a polarity similar to that of ethanol. After inclusion, the host protects the probe from the solvent molecules, external quenchers, and oxygen. Within the nano-sized cavity of the CD, rotational motion of the guest also gets restricted. All these factors contribute to the enhancement of the fluorescence intensity of the guest fluorophore on encapsulation.

CDs have been used as a cyclic component in the construction of supramolecular architectures. CDs are found to form inclusion complexes with various hydrophobic and hydrophilic polymers with high selectivity producing polymer induced CD-nanostructures. There are many examples of formation of small organic molecule induced CD nanotubes and nanostructures. First of the latter kind was reported by Li et al. on 1990. After that many reports appeared on probe induced CD nanotubes. Non-covalent interactions, such as H-bonding, hydrophobic and van der Waals are supposed to be the driving forces in the formation of probe induced CD supramolecular aggregates. Depending on its size, a CD molecule is capable of accommodating one or two guest molecules in most cases. The CD nanotubes are subject of great interest because of their novel properties and potential applications in a variety of areas, especially in drug delivery. In particular, for organic nanotubes, the precise functionalization and interconnection of the building blocks suggest that they can exhibit not only unprecedented architectures but also valuable functions for applications in electronics and biomedicines. The properties of CD nanotubes can be modulated by functionalization and choosing proper guest molecules.

**Inclusion in lipid vesicles**

Lipid vesicles are complex combinations of water and lipid molecules. Lipid vesicles may contain other additional components, e.g., salts, carbohydrates, cholesterol, small peptides, etc. In cell membranes the structure of lipid layer is more complex; there it may contain many receptors, proteins, etc. To mimic the biological conditions in synthetic lipid vesicles, carbohydrates and salts are added quite often. Grossly, lipid vesicle can be explained as water filled bladder immersed in water. Lipid vesicles or liposomes have three main regions: (i) inner water pool, (ii) hydrophobic bilayer and (iii) two interfacial zones; one towards the inner water pool and the other towards the bulk water. However, understanding of the detailed structure at the molecular level is limited by the lack of high-resolution three-dimensional structures of lipid vesicles. It is extremely difficult to crystallize membrane-bound molecules for diffraction studies. Due to these limitations, fluorescence spectroscopic technique is regularly used as a very important tool to discover different properties of lipid vesicles by analyzing the changes in the dynamics of interactive fluorophores.

Different analytical techniques and molecular dynamics (MD) simulations show that all lipids have two phases, a solid crystalline state at a lower temperature and a gel state above a certain temperature, called glass transition temperature. Gel-state has more fluidity over the solid crystalline state. The fluidity of the non-polar bilayer increases with a decrease in chain length of lipids. Fluorescence studies showed that the polarity of water entrapped inside the pool of lipid vesicle is quite small and is comparable to that of methanol. MD simulation indicates that above the glass transition temperature each lipid molecule is hydrogen bonded to few water molecules which form an inner hydration shell of the polar head group of the lipids. Majority of the lipid molecules remain connected by water bridges. There is a sharp transition in relative permittivity from ~80 to ~1 at the interfacial region of bulk water and lipid bilayer within less than 1nm distance.

**Some special experiments**

**Photophysical studies of some indoloquinoline derivatives in solvents of different polarity and the hydrophobic nanocavities of cyclodextrins**

Cryptosanguinolentines is regarded as one of the most important indoloquinoline alkaloids. These compounds can be conveniently synthesized through photochemical cyclization and Fischer indole synthetic routes. Such alkaloids can intercalate in DNA double helix and may inhibit DNA replication and transcription. Some of their N-methyl derivatives show important antimicrobial and cytotoxic activities. Three cryptosanguinolentines, viz., 5-methyl-5H-indolo[3,2-c]quinoline (MIQ), 8-chloro-5-methyl-5H-indolo[3,2-c]quinoline (CMIQ) and 2,8-dichloro-5-methyl-5H-indolo[3,2-c]quinoline (DCMIQ) were studied in CDs to observe alterations in their photophysics. Representative structures of these compounds are demonstrated in Scheme I.

The absorption spectra of the three compounds do not show much change on addition of CDs to their aqueous solutions. Their emission spectra are broad in aqueous solution due to the existence of two solvated
species at room temperature. The band ~470 nm is attributed to the flank-solvation of the molecules and that ~440 nm is due to the centrally solvated chromophoric species. On addition of α-CD, having the smallest cavity diameter in the CD family, to MIQ in water, no appreciable change in the fluorescence spectrum was observed. However, CMIQ and DCMIQ had a profound effect due to the interaction as shown in Figure 1a, b and c. These compounds being flanked by hydrophobic chloro groups promote interaction with α-CD.

The photophysical manifestation changed completely for the three fluorophores on addition of β-CD (Figure 1b). An initial quenching of the fluorescence of the zwitterions followed by a small recovery is recorded. The extent of quenching of fluorescence is greatest for DCMIQ and lowest for CMIQ. Quenching of MIQ fluorescence is intermediate among the three saturating at higher concentrations of β-CD. Recovery of fluorescence starts after addition of a certain amount of β-CD into the solutions. On application of γ-CD to the aqueous solutions of the fluorophores, a progressive quenching of the fluorescence of the zwitterions of CMIQ and DCMIQ is observed (Figure 1c). MIQ does not get remarkably affected by γ-CD.

This effect on the photophysics of the fluorophores is presumably due to the dual effect of the size of the nanocavities of the CDs and their structural features. It is noted that the zwitterionic forms of the molecules in the excited state play the most important role in the host-guest chemistry. The zwitterions may form dimers through Coulombic interaction thus reducing the fluorescence yield of the monomeric zwitterions. Scheme II demonstrates the stacking motifs of MIQ, CMIQ, and DCMIQ zwitterions. The nature of encapsulation of the molecules depends on the hydrophobicity imposed by the presence of the chloro functionalities in the compounds.

CMIQ fluorescence undergoes a remarkable progressive enhancement with an increase in α-CD concentration.
The CMIQ excimer shows the presence of two chloro groups projecting from opposite sides (Scheme II). These hydrophobic substituents may lead to better encapsulation by the hydrophobic α-CD cavities as shown in Scheme III. The intensity of the 440 nm band increases. The encapsulation of the -Cl group of CMIQ makes it more electron withdrawing. This reduces the electron density on the N-centre of CMIQ, which in turn triggers the dynamics of more zwitterion formation as per the laws of chemical equilibrium. This phenomenon increases the fluorescence intensity of the zwitterions. DCMIQ suffers a sharp initial quenching of fluorescence at lower concentrations of β-CD followed by a rapid partial recovery. In this case, the excimer tries to get encapsulated inside the CD cavity initially followed
by the generation of the entropically favourable 1:2 complex at higher host concentrations. The steep quenching is presumably due to better encapsulation reinforced by the presence of more chloro groups in the stack. CMIQ forms excimer and goes inside the γ-CD cavity. Enhancement in the encapsulation of the excimers inside the CD cavities provides more stability to the stack. On the other hand, better stacking is promoted by the better formation of the zwitterions and their period of existence in the excited state. The molecules inside the cavity dwell with some water molecules solvating the chloro groups at the flanks thus lowering the electron withdrawing capability of the -Cl groups. DCMIQ has two chloro groups on its opposite flanks that provide better stability to the zwitterions, which in turn may lead to the formation of excimers that get encapsulated by γ-CD from both sides leading to higher quenching of the zwitterionic fluorescence.

Small molecule induced architecture of cyclodextrin aggregation

Self-aggregation of cyclodextrins (CDs) in water is a long-standing mystery. Some reports have been published on this using the concept of self-aggregation of CD with or without the aid of an externally added compound. In general, native CDs can form 200-300 nm long aggregates in water depending on the type of CD.

The CDs prefer to line up in ideally parallel or staggered parallel arrangement with quadrupolar character. The hydrophobic inner cavities of the truncated cone-like CDs with six-, seven-, and eight-member sugar residues constituting α-, β-, and γ-CDs, have 4-8 Å diameters. A single CD cavity can hold one or more guest molecules constrained to size. The structure of guest and host molecules is important in the formation of CD superstructures. To investigate this phenomenon, the model fluorophore considered here is a potent G-quadruplex DNA binding small molecule, bis-phenylethynyl amide meta-linked to 2,6-pyridine (BPEAP), as shown in Scheme IV.

Two distinct absorption peaks are present for BPEAP in water at 260 and 350 nm. The addition of CD to BPEAP solution in steps of 1 mM does not show any noticeable change in the absorption spectrum. On using Job’s method of continuous variation to determine the stoichiometric ratios of the hosts to the guest, bimodal plots were obtained as shown in Figure 2a, b and c. Job’s plot is generally used to determine the nature of the interaction between two or more species during complexation. At very low CD concentrations, where the proportion of the guest compound is higher than the host, 2:1 guest-host complexation is preferred, whereas, when the proportion reverses, 1:3 guest-host interaction is favourable. Insertion of the hydrophobic side-chains of two BPEAP molecules inside the host cavity at lower host concentration is responsible for such stoichiometry. On the other hand, when host concentration

Scheme IV — Structure of bis(phenylenethynyl) amides meta linked to 2,6-pyridine (1) (abbreviated as BPEAP), and possible equilibrium with its ionic counterpart (2) (Reproduced from J Phys Chem C, 115, 2011, 20970).
increases, each carboxamide side-chain and a central pyridine ring of BPEAP get encapsulated with host molecules to yield 1:3 guest-host complexes.

BPEAP shows two emission peaks at ~ 380 nm and ~ 490 nm due to the neutral and the ionic species (Figure 3). The more solvated ionic species fluoresces at higher wavelength compared to the neutral one. A 50 nm hypsochromic shift is observed at the emission maximum (490 nm) of BPEAP on the addition of α-CD accompanied by appreciable intensification. On comparison of the intensities of the two peaks in the presence of the different CDs, a considerable enhancement in the ratio was observed when BPEAP was treated with α-CD. This indicates that the ionic species of BPEAP is affected considerably due to the encapsulation over the neutral. On the contrary, a significant decrease in the ratio is observed on addition of β- and γ-CDs to BPEAP suggesting differential interaction.

The differential encapsulation of BPEAP in the three CDs depending on their cavity size was modelled through a scheme based on the change in fluorescence emission that reflected compactness of the binding of the fluorophore. Scheme V shows the proposed structural motifs in each case. The structures were supported by atomic force microscopy images (Figure 4).

**Light-induced dynamics of a charge transfer probe in lipid vesicles**

Biological membranes are complex assemblies of lipids and proteins that allow many important cellular functions. The cell membrane of almost all living organisms and other subcellular structures is constituted of a lipid bilayer that acts as a barrier to secure the positions of ions, proteins and other molecules and acts as a solvent to membrane proteins. Amphiphilic phospholipids organize as bilayer in water and are stabilized using intermolecular forces between the polar head groups and hydrophobic interaction between the hydrocarbon chains of the fatty acid. The assemblies include water molecules for solvation. The low polarity of the hydrophobic cores created by the fatty acid chains yields dielectric constant values around 2–4 for this region of the lipid bilayer. Water exchanges rapidly between the exterior and the interior of lipid vesicles, implying the presence of water molecules within the hydrophobic core of the lipid bilayer. The "S\textsubscript{N}2" carbonyl group is more hydrated than the "S\textsubscript{N}1" in lipids. A representative structure of DPPC showing S\textsubscript{N}1 and S\textsubscript{N}2 is given in Scheme VI.

Herein, a reporter molecule, trans-2-[4-(dimethylamino)styryl]benzothiazole (DMASBT) as shown in Scheme VII, in considered to illustrate the properties of lipid vesicles in an aqueous environment. DMASBT undergoes twisted intramolecular charge transfer (TICT) and shows a structureless emission band at a wavelength higher than that for the locally excited (LE) species in polar solvents. DPPC has been used to prepare small, large and giant unilamellar vesicles (SUVs, LUVs and GUVs, respectively). In each of them, the size of the internal water pool differs and provides an excellent platform to study the distribution of a probe that is hydrophobic in the ground state but becomes hydrophilic when excited.

![Figure 2](image-url) — Absorbance values at 261 nm have been used in the Job’s method to generate the Job’s plots for (a) α-, (b) β- and (c) γ-CDs (Reproduced from J Phys Chem C, 115, 2011, 20970).
DMASBT shows intense structureless emission band peaking at 535 nm in aqueous solution due to TICT and a structured LE band at 420–450 nm. This fluorophore is extremely sensitive to the environment polarity. Addition of lipid vesicles in an aqueous solution of DMASBT produces a large hypsochromic shift of the emission band and profuse enhancement in fluorescence intensity as shown in Figure 5. In SUVs, the emission maximum of DMASBT shifts to 471 nm, i.e., a blue shift by about 65 nm from that in water (Figure 5a). This indicates less penetration of water into the hydrocarbon microenvironment in SUVs that lowers the energy of the non-radiative decay pathways.

The position of the emission maximum of the probe in the presence of lipid vesicles indicates that the relative permittivity of the microenvironment is about 6–10 which is possible only if the molecule is present in the hydrophilic–hydrophobic interface of the lipid vesicles where a sharp transition of relative permittivity occurs within 1 nm. Thus, it is apparent that DMASBT may exist in the $S_N^1$ region of both the interfaces of the lipid vesicles on excitation. However, the position of fluorescence maximum indicates that most likely the molecule is present in the relatively more non-polar region in SUVs compared to LUVs and GUVs (Figure 5b and Figure 5c).

On excitation, DMASBT undergoes charge separation and moves towards the zwitterionic DPPC head groups at the inner as well as the outer aqueous zones. Among the three major regions in the lipid membrane, the interfacial region is recognized by...
Scheme V — Different proposed motifs of aggregation of the three different CDs with BPEAP based on the spectral evidences (Reproduced from J Phys Chem C, 115, 2011, 20970).

Figure 4 — Atomic force micrograph of BPEAP induced (A) α-, (B) β-, and (c) γ-CD aggregates (Reproduced from J Phys Chem C, 115, 2011, 20970).
Scheme VI — Representative structure of 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) showing the $S_{\text{N}1}$ and $S_{\text{N}2}$ carbonyl groups.

Scheme VII — Representative structure of DMASBT. $\phi$ shows twisting of the $-\text{N(CH}_3)_2$ moiety.

Figure 5 — Fluorescence spectra of DMASBT with increase in the concentration of (a) SUVs, (b) LUVs and (c) GUVs. The direction of the arrows indicates enhancement in the concentration of lipid vesicles from 0–100 mM. The excitation wavelength is 360 nm (Reproduced from Soft Matter, 8, 2012, 10178).
unique motional and dielectric characteristics in contrast to the bulk aqueous phase\(^2\). This specific region of the membrane exhibits a slow rate of solvent relaxation and is also known to participate in intermolecular charge interactions and hydrogen bonding through the polar headgroup\(^3-^6\). Moreover, the \(S_\text{c}^2\) carbonyl group is more hydrated than the \(S_\text{c}^1\). In the excited charge separated state, DMASBT experiences this heterogeneity and gets distributed accordingly.

**Conclusion**

Three different instances have been presented here in perspective to photophysical modulations of biologically potent small molecules in biocompatible microheterogeneous environments created by cyclodextrins and lipid vesicles. In the first example, the interaction of MIQ, CMIQ, and DCMIQ with \(\alpha\)-, \(\beta\)- and \(\gamma\)-CDs indicate that the zwitterions predominating in the excited state of the molecules have pronounced behavioural difference in CD environments. Limited confinement by \(\alpha\)-CD cannot change the fluorescence behaviour of MIQ remarkably, but on the addition of chloro group/s to the indolyl and the quinolinoyl phenyl rings leads to a profuse enhancement in fluorescence. Tight confinement of the generated excimers of the compounds inside the \(\beta\)-CD cavities demonstrates initial quenching of the zwitterionic fluorescence followed by a recovery. \(\gamma\)-CD provides more space to the excimers and thus loses the fluorescence from the monomeric zwitterions. We have demonstrated simple applications of CD cavities of different sizes on indoloquinoline derivatives that are potentially used as anticancer drugs. The formation and stability of the formed excimers from the molecular zwitterions could effectively be controlled by using different concentrations of CDs of different dimensions. The dimension of the hydrophobic interior of the CDs appears to act as the driving force towards excimer formation.

In the second example, we have observed that the structure of the guest and size of the host (CD) determine the motif of guest-induced host aggregation. The host size is responsible for the nature of the aggregation. Particular aggregation behaviour can be monitored quite explicitly using fluorescence spectroscopy if the guest molecule fluoresces. A biologically potent guest that can bind to the G-quadruplex DNA and contains three potential sites for host encapsulation has been chosen. A schematic for host-guest binding has been proposed based on spectral evidence.

It is also observed that DMASBT is a sensitive and suitable fluorescent probe for determining the heterogeneous environments of lipid vesicles of different sizes. Results show that the hydrophobic DMASBT molecules exist in the less-polar hydrophobic bilayer of the vesicles in the ground electronic state and on charge separation in the excited state, protrudes differentially toward the bulk water and the inner aqueous core of the lipid vesicles. The TICT species depends upon the size of the aqueous pool in the lipid vesicles and on the penetrated water molecules at the interface. Since lipid vesicles mimic the environment of biological cells, our findings will reinforce the understanding of the distribution of charge transfer molecules in such environments.

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