NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

pH Dependent Supramolecular Recognition of Dapoxyl Sodium Sulfonate with 2-Hydroxypropyl *β*-Cyclodextrin: An Application Towards Food-Additive Formulation

*Kaushik Pal, Falguni Chandra,‡ Suman Mallick,‡ Apurba L. Koner**

Department of Chemistry, Indian Institute of Science Education and Research Bhopal, Bhopal, India. **Corresponding Author:** * E-mail: akoner@iiserb.ac.in, Fax: +91-755 6692 392; Tel: +91-755 6692 376. **Author information**: ‡These authors contributed equally.

KEYWORDS: Dapoxyl dye, 2-Hydroxypropyl *β*-cyclodextrin, Fluorescence, Displacement assay, Food-additive.

ABSTRACT: pH dependent host-guest complexation of Dapoxyl Sodium Sulfonate (DSS), an intramolecular charge transfer dye with 2-Hydroxypropyl beta cyclodextrin (HP-*β*-CD) has been investigated. Complexation of DSS with HP-*β*-CD has been studied at four different pH using steady-state and time-resolved spectroscopy. Cyclodextrin encapsulation alters the acid-base property of DSS and results in host-induced deprotonation. A large fluorescence enhancement of DSS was observed upon HP-*β*-CD binding at different pH and it enabled us to evaluate the pH dependent affinity of DSS with HP-*β*-CD. Binding affinity for non-protonated DSS was much higher when compared to its protonated state. Subsequently, the encapsulation induced fluorescence enhancement at *ca.* pH 7.0 has been implemented for developing a fluorescence displacement principle for assaying binding affinity of food-additives namely monosodium glutamate, *trans*-ferulic acid, *p*-coumaric acid, gallic acid and its methyl, ethyl and propyl ester derivatives with HP-*β*-CD. Other than monosodium glutamate, all other tested food-additives show preferential binding to the hydrophobic cavity of HP-*β*-CD. Therefore, this quick method for assaying the binding affinity of food additives with water-soluble macrocyclic host molecule can offer better food processing ability, control activity, and stability.

1. Introduction:

There is a long-lasting and growing concern on the unrestricted use of pesticides, antibiotics, and food-additives on human life.[1, 2] Uncontrolled use of antibiotics in both developed and developing countries is one of the definite cause for antibiotic resistance of bacteria. Similarly, excessive use of food-additives also causes multiple health problems in terms of hormonal disorder, indigestion, ulcers in intestines and ultimately leading to colon cancer.[3-7] Poor aqueous solubility and long-term stability of food-additives demand their excessive use and ultimately causes accumulation and adverse effect in biosphere.[8-10] In many cases, degradation products originating from these molecules also cause an unhealthy effect on human life.[11, 12] Therefore, there is a constant effort to increase the solubility, overall stability, and control release of food additives. Polyphenol containing compounds are well-known food additives and important biologically active molecules, generally found in plants as a secondary metabolites.[13, 14] In fact, these polyphenol-based compounds have been widely used as food additives, due to their favourable antioxidant, antimicrobial, and taste enhancing properties.[14, 15] Distinctly, good hydrogen-bonding ability and aromaticity of such phenolic compounds enable them to act as free-radical scavengers by forming a stable aryloxyl radical. Importantly, such stabilization of these radicals in combination with other functional groups enhances the antioxidant activity.[16] In addition to their primary antioxidant activity, phenolic compounds are known to modulate carcinogenesis. Gallic acid and its alkyl ester derivatives are employed as antioxidant in food additives, which prevents oxidative damage induced by reactive oxygen species (ROS).[13, 16] Moreover, gallic acid derivatives are known to cause apoptosis in tumour cell lines and to inhibit lymphocyte and estrogen.[17] The dependence of their rate of incorporation into cell directly related to their lipophilicity.[18, 19] The lipophilicity of any compound can be modulated mainly in two different ways: covalent functionalization by synthetic modification or *via* introducing intermolecular interactions with other benign additives.[20, 21]

Encapsulation of small molecule food-additives by non-toxic, and water-soluble macrocyclic host can reinforce their bioavailability and solubility by modulating physical and chemical properties.[22] Water-soluble macrocyclic host molecules can encapsulate sparingly water-soluble drug, antibiotic, and food-additives as a consequence of weak intermolecular interactions. Such encapsulation causes modulations of their solution properties and triggers the activation of a drug from its

proactive form to its active state.[23, 24] Supramolecular drug-macrocycle complex has already been shown great promise for their usefulness in targeted drug delivery applications.[25-28]

High water solubility of cyclodextrins (CDs) makes them suitable for the formulation of water-insoluble to sparingly-soluble drugs, antibiotics, hormones *etc.*[21, 27] CDs are a family of macrocyclic compounds composed of sugar units connected by 1,4-glycosidic linkage and depending on the number of sugar units present in the cyclic structure, they are dubbed with different names. Alpha-, beta- and gamma-cyclodextrin consists of six, seven and eight number of sugar units respectively.[29] The hydrophobic cavity of CD provides a new environment to the encapsulated guest molecule, which is significantly different from the exterior bulk aqueous environment. This unique cavity can modulate chemical and physical properties of encapsulated guest molecule.[30]

Recently, we have reported the solvent dependent photophysical properties of Dapoxyl Sodium Sulphonate (DSS, see Fig 1), an intramolecular charge transfer (ICT) dye [31-33] and its pH dependent complexation with α, β, and γ-CD [25, 34] In our earlier studies, we have also demonstrated that the encapsulation causes modulation of acid-base properties of DSS and relocation of DSS to a less polar hydrophobic cavity of γ-CD compared to bulk aqueous phase resulted a remarkable fluorescence enhancement originating from the charge transfer state.[25] Further, we have also used γ-CD induced fluorescence enhancement for displacement assay for estimating the binding affinities of small molecule drug and antibiotics.[25]

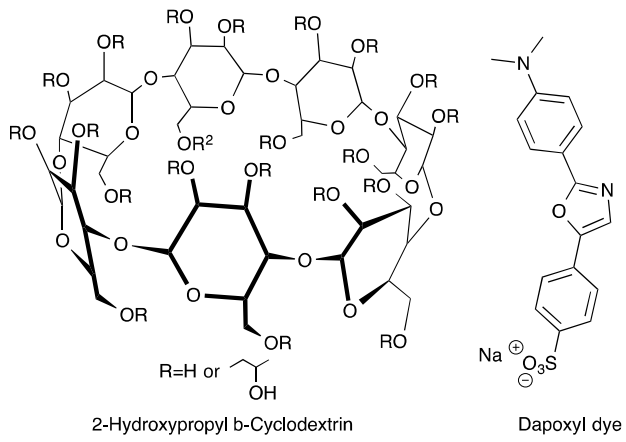

Figure 1. Chemical structures of 2-Hydroxypropyl β-cyclodextrin (HP-β-CD) and Dapoxyl Sodium Sulfonate (DSS).

Herein, we have further extended our study of DSS complexation with one of the highly water-soluble cyclodextrin derivative namely 2-Hydroxypropyl β-cyclodextrin (HP-β-CD, for structure see Fig. 1) using UV-Vis., steady-state and time-resolved fluorescence spectroscopy. Much stronger binding affinity of DSS with highly water-soluble HP-β-CD as compared to parent β-CD was observed. Differential pH dependent binding strength of DSS with HP-β-CD also reflected in a complexation-induced deprotonation by one logarithmic unit. A large fluorescence enhancement of DSS upon encapsulation with HP-β-CD was observed and this was utilized to develop a fluorescence-based displacement assay to assess the binding affinities of seven frequently used food additives (monosodium glutamate, *trans*-ferulic acid, *p*-coumaric acid along with gallic acid and its methyl, ethyl and propyl ester) with HP-β-CD at physiological pH.

2. Materials and Method:

2.1 Materials: DSS was purchased from Invitrogen (USA). HP-β-CD, *trans*-ferulic acid, and *p*-coumaric acid were from Sigma-Aldrich, USA. All other food additives were purchased from TCI chemicals, Japan. All the chemicals were used as received without performing any further purification.

2.2 Steady state absorption and fluorescence measurement: Steady state absorption measurement was done by Shimadzu UV-Spectrophotometer 1800 (UV probe 2.42 software) using 1 cm path length quartz cuvette. All steady state fluorescence measurements were carried out using HORIBA Jobin Yvon Fluoromax-4 fluorimeter. A dilute solution of DSS (4 μM) was taken for all the measurements to keep the absorption value low in order to avoid the inner filter effect. Binding titration experiments were carried out at four different pH *ca.* 2.0, 4.0, 7.0 and 9.0 where pH was adjusted by using dilute HCl and NaOH solutions. To avoid dilution effect in the titration, HP-β-CD stock solutions were prepared in the same DSS solution and pH was adjusted accordingly. Displacement assay with food-additives was performed at physiological pH *ca.* 7.4 with HP-β-CD using different concentration of food additives. All the experiments were carried out at ambient temperature (298 K).

2.3 Time resolved measurement: Time resolved fluorescence measurements were performed using a Hamamatsu MCP photomultiplier (R-3809U-50). A time-correlated single photon counting (TCSPC) setup consists of an Ortec 9327 pico-timing amplifier was used. Pulse laser was used as an excitation (λ_{ex}=375 nm) source with fwhm ~167 ps. The instrument response function (IRF) was collected using a dilute suspension of Ludox (colloidal silica, purchased from Sigma). The emission polarizer was positioned at magic angle (54.7º) with respect to excitation polarizer. Single and multi-exponential fitting function was applied by iterative deconvolution method using supplied software DAS v6.2. General form of the fitting function was:

$$I(t) = I(0) \Sigma a_i \exp(-t/\tau_i)$$

Where I (t) and I (0) are the fluorescence intensity at time t and 0 respectively, t is time and a_i and τ_i are the contributing amplitude and its corresponding lifetime. Quality of the fitted data was judged from the reduced chi-squared value (χ^2). All the measurements were carried out at ambient temperature (298 K).

2.4 Displacement assay titration: Displacement assay was performed at pH 7.4 using a 96-well microtiter plate containing a total of 200 μL solution of 5 mM HP-β-CD and 4 μM DSS and required amount of food additive solutions having same concentration of DSS and HP-β-CD. We used 96 well plate purchased from Globe Scientific, USA and measured the fluorescent intensity at 550 nm using bottom read manner upon excitation at 340 nm using H1M synergy microplate reader from Biotek instrument, USA.

2.5 pH titration, binding titration and their analysis: pH dependent ground-state absorption properties of 4 μM DSS and in case of complex a 4 μM DSS and 10 mM HP-β-CD were used to determine the acid-dissociation constant values. The pH dependent binding constants of DSS with HP-β-CD were obtained from the data fitted with 1:1 binding equation using nonlinear fitting procedure of the Pro Fit 6.2.9 software as reported earlier by Nau and co-workers.[35, 36] In all the measurements estimated error was below ±10%.

3. Result and Discussions:

3.1 UV-Vis and fluorescence titration of DSS with HP-β-CD at pH 7.0

In order to study the interaction between DSS and HP-β-CD in ground state at pH 7.0, a UV-Vis titration was performed. A pH adjusted solution of 4 μM DSS was titrated with a concentrated solution of HP-β-CD prepared in same concentration of DSS at same pH to avoid the dilution and pH change effect. The experiment results in a gradual hyperchromic shift (OD 0.105 to 0.120) in absorption associated with a significant hypsochromic shift (20 nm) in absorption maxima (Fig. 2a).

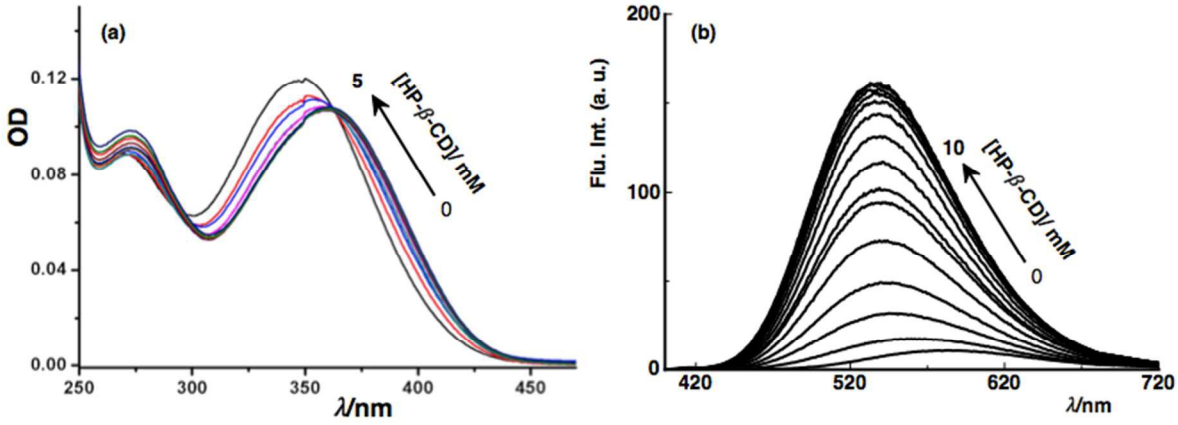

Figure 2. UV-Vis and fluorescence titration of 4 μM DSS with HP-β-CD at pH 7.0 (a) complexation induced changes in the absorption spectra of DSS with gradual addition of HP-β-CD, (b) fluorescence enhancement of DSS upon complexation with gradual addition of HP-β-CD up to 10 mM

A clearly visible isosbestic point at 365 nm indicates formation of 1:1 HP-β-CD-DSS complex. This is in accordance with 1:1 binding of the parent β-CD.[34] The small hyperchromic shift followed by a large hypsochromic shift indicates that the encapsulation of DSS by HP-β-CD does not alter the allowance of electronic transition probability but does stabilize the HOMO, from where the electronic transition occurs. On the other hand, the fluorescence titration of DSS with the increasing concentration of HP-β-CD using steady state fluorescence spectroscopy results a huge fluorescence enhancement associated with *ca.* 60 nm blue shift. A typical fluorescence titration of DSS with increasing concentration of HP-β-CD at pH 7.0 is shown in Fig. 2b. Encapsulation of DSS by HP-β-CD protects DSS from outside solvent environment and the collision with other quenchers like dissolved oxygen and impacts immensely on the excited-state emissive properties (*vide infra)*. For

any fluorophore in solution, one of the major excited-state deactivation pathways arise mainly from the interaction and collision between fluorophore and solvent molecules *via* dipole-dipole interaction. In fact, excited state of an ICT dye like DSS has high dipole moment, causes obvious interactions with solvent dipoles and deactivation of excited-state *via* non-radiative processes.[37] Hence, the encapsulation of DSS by HP-*β*-CD not only protects it from the collisions with solvent molecules and but also offers a unique local environment to DSS in terms of polarity and results in a huge enhancement (*ca.* 25 times) in the fluorescence intensity.

3.2 Effect of HP-*β*-CD on the acid-dissociation constant of DSS

Supramolecular recognition is often reinforced by the complementary size and shape between the host cavity and the guest, whereas the weak intermolecular forces like dipole-dipole, dipole-induced dipole, charge-dipole and H-bonding interaction in cooperation strengthen the binding strength. In case of CD, primary and secondary hydroxyl groups residing on the periphery can act both as H-bonding donor and acceptor.[29] Therefore, being a polar protic group it is a suitable partner for dipolar interactions and delivers differential binding interactions with different prototropic form of the encapsulated guest molecule. As a result, this preferential stabilization of one prototropic form of the guest molecule than the other form causes a shift in the protonation-deprotonation equilibrium.[23, 38-40] Therefore, depending on the extent of the stabilization of different forms, there will be a shift in the acid-dissociation constant.[24] The shift can be in both sides of the pK_a value.[41] In this study, in the studied pH range, the guest DSS can remain in mainly two different prototropic form monoprotonated and non-protonated. In aqueous solution, the pK_a value of DSS is around 4.2.[31] In fact, this protonation effect can easily be monitored spectrophotometrically as protonated and deprotonated form of DSS have distinct absorption spectral features. Indeed, the pH dependent UV-Vis. spectra of DSS in absence and presence of HP-*β*-CD (Fig. 3a) shows the evolution of 305 nm band and attenuation *ca.* 380 nm band occurs through one isosbestic point at 337 nm allow us to determine the pK_a values in free and in encapsulated state.

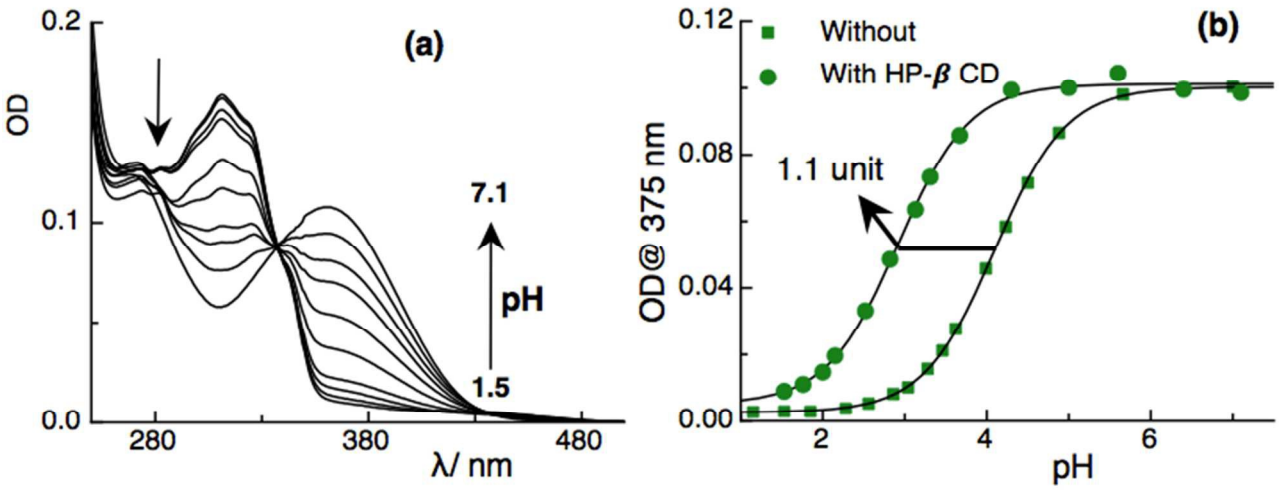

Figure 3. pH titration of DSS (a) pH dependent UV-Vis spectra of 5 μM DSS, (b) pH dependent plot of optical density of DSS in presence and absence of 10 mM HP-β-CD; arrow indicate 1.1 units pK_a shift upon encapsulation.

Interestingly, the presence of distinct isosbestic point clearly indicates the presence of only two prototropic states both in free and as well as in the encapsulated state. The pH titration of free DSS and DSS•HP-β-CD results in a sigmoidal shaped graph. Eventually, fitting of these sigmoidal curves with two-state equation provides us the pK_a value of DSS in free and in complexed state.[40] A negative shift of 1.1 units in the pK_a value was obtained. This indicates a destabilization of the protonated state of DSS in the HP-β-CD cavity in comparison to the non-protonated form. Agreeably, this because of the fact that hydrophobic CD cavity disfavour encapsulation of positively charged molecule over neutral molecule and such preferential binding causes negative one unit shift in the pK_a value of DSS. Indeed, this pK_a shift also affects on the pH dependent fluorescence properties and binding affinity of encapsulated DSS.

3.3 pH dependent binding of DSS with HP-β-CD

The driving force for encapsulation of guest molecules by CD originates mainly due to two types of intermolecular forces. The non-polar hydrophobic cavity offers dispersion forces and the hydroxyl groups projected at the entrance of the cavity enforce the possibility of H-bonding with the polar part of the encapsulated guest. The incorporation of hydroxypropyl groups in both primary and

secondary surface of *β*-CD strengthens both hydrophobic and H-bonding interactions with guest molecules. Hence, HP-*β*-CD will preferentially encapsulate a hydrophobic molecule containing a negatively charged moiety over positive charge. The pK_a of DSS is 4.2, accordingly, it is likely that at pH 2.0 and pH 4.0 the guest DSS will be fully or partially protonated *i.e.,* with an extra positive charge, whereas at pH 7.0 and 9.0 the guest is not protonated *i.e.,* devoid of any extra positive charge. Therefore, HP-*β*-CD is likely to encapsulate non-protonated DSS with stronger affinity within compared to the protonated or partially protonated DSS. Indeed, fluorescence titration of DSS with HP-*β*-CD at four different pH *ca*. 2.0, 4.0, 7.0 and 9.0 confirm our hypothesis. In acidic pH range, below pK_a value, DSS exhibits two emission bands, one maxima is around 385 nm corresponds to the locally excited state and the other one with maxima around 585 nm in agreement with the ICT state (Fig. 4a and 4b). At pH above pK_a value of DSS, only one fluorescence band due to ICT state has been observed (Fig. 2b and 4c). This fact reveals that the protonated form can have a radiative relaxation to the ground state from locally excited state but that is absent in deprotonated form.

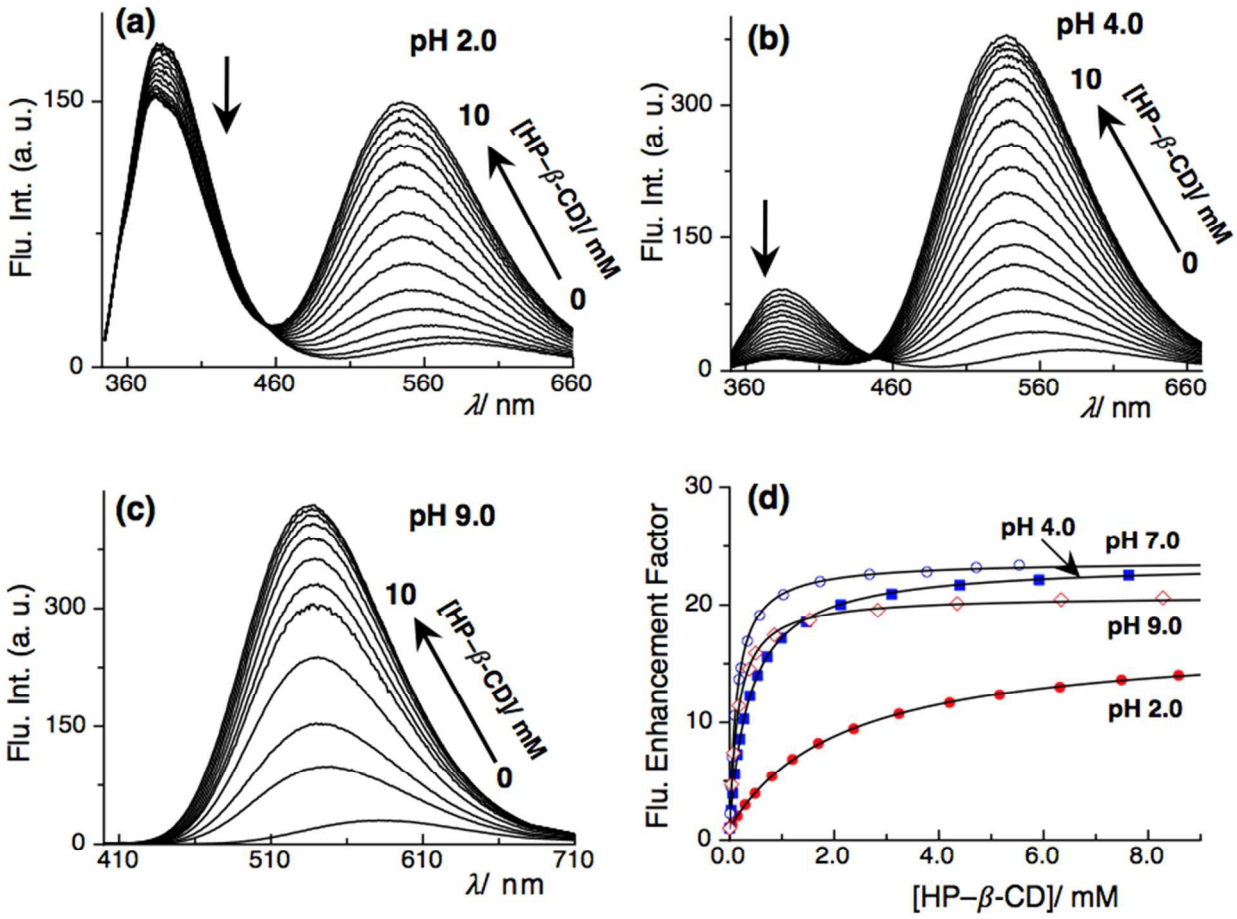

Figure 4. Binding titration of DSS with increasing concentration of HP-*β*-CD in water (a) at pH 2.0, (b) at pH 4.0 and (c) at pH 9.0, (d) relative fluorescence intensity change of DSS in different pH upon HP-*β*-CD complexation is plotted against HP-*β*-CD concentration and fitted using 1:1 binding equation.

The titration at pH *ca*. 4.0 which is close to the pK_a of DSS (pK_a is 4.2) shows two bands without HP-*β*-CD but the band due to emission from locally excited state diminished with the increase of the concentration of HP-*β*-CD, which is due to the host dependent deprotonation of the guest DSS. Not only the enhancement of fluorescence intensity or host promoted deprotonation of the species, HP-*β*-CD also provides a relatively nonpolar environment to the guest as observed in the case of previously studied examples. The shift of the fluorescence maxima from 585 nm to 550 nm confirms relocation of DSS from water to the hydrophobic cavity of HP-*β*-CD. The fluorescence

enhancement of DSS upon HP-*β*-CD encapsulation was utilized to determine the binding affinity. The cavity volume offered by both the parent *β*-CD and HP-*β*-CD is similar. According to our previous report based on the Job's plot, we showed that only one DSS molecule can be accommodated by *β*-CD.[34] Fluorescence intensity of DSS at a particular wavelength was plotted against the concentration of HP-*β*-CD and fitted with a 1:1 equation.[35] A comparison of pH dependent binding between DSS and parent *β*-CD and HP-*β*-CD are summarized in Table 1.

Table 1: pH dependent binding constants, fluorescence lifetime of DSS and effect of CD encapsulation

Studied pH		**pH 2.0**	**pH 4.0**	**pH 7.0**	**pH 9.0**
K/ M^{-1} [a]	*β*-CD[b]	400	925	2970	2910
	HP-*β*-CD	466	2940	7130	6120
$\tau_{□□□}$/□□ (χ^2)	□□□ [b]	1.5 (1.2), 2.0 (1.2) [c]	1.6 (1.2), 1.9 (1.1) [c]	1.9 (1.2)	2.1 (1.1)
	β-CD[b]	1.5 (1.2), 2.2 (1.1) [c]	1.5 (1.1), 2.2 (1.1) [c]	2.5 (1.1)	2.5 (1.1)
	HP-*β*-CD	2.0 (1.1), 3.1 (1.1) [c]	2.6 (1.1), 3.7 (1.1) [c]	3.7 (1.1)	3.7 (1.2)

[a] ±10% error, [b] taken from ref. [25, 34], [c] measured at the emission maxima originated from locally excited and charge transfer state ref 33

It is clearly evident from table 1, that the pH dependent binding strength between DSS and HP- *β*-CD is significantly higher as compared to the same with *β*-CD. This result clearly indicates that hydrophobic interaction between DSS and HP-*β*-CD is the most dominant interaction. Thus, the pH dependent binding results and its comparison further prompt us to have a look into the excited state properties using time-resolved spectroscopy.

3.4 Fluorescence lifetime enhancement of DSS upon encapsulation with HP-*β*-CD

Upon irradiation with an appropriate energy of light, an organic fluorophore undergoes an electronic transition into higher energy electronic level and reside in the higher energy state for certain time before its deactivation to ground state. In fact, fluorescence occurs from an excited

electronic state to the lower energy ground state having same spin state of the molecule *via* a spin allowed and radiative de-activation process. Therefore, the average time spent in the excited state of the fluorophore is called lifetime of that excited state. Generally, how long a molecule will stay in the excited state is governed by several parameters. In most cases, the polarity of the medium, possibility of H-bonding, presence of quencher molecules, excited state energy or proton transfer, temperature, *etc.,* have a prominent role on the fluorescence lifetime. Macrocyclic host molecules like HP-*β*-CD can protect the excited state of DSS from collisions with surrounding solvent molecules, which also acts as quencher. Other than this, conformational flexibility of encapsulated DSS can be restricted upon encapsulation into HP-*β*-CD cavity. Eventually, such protective shielding and conformational restriction by HP-*β*-CD can alter radiative rates, which in turn affect on the fluorescence lifetime. To strengthen the evidence of the binding event between DSS and HP-*β*-CD a time dependent fluorescence study also has been performed at different pH (Fig. 5, ESI S1).

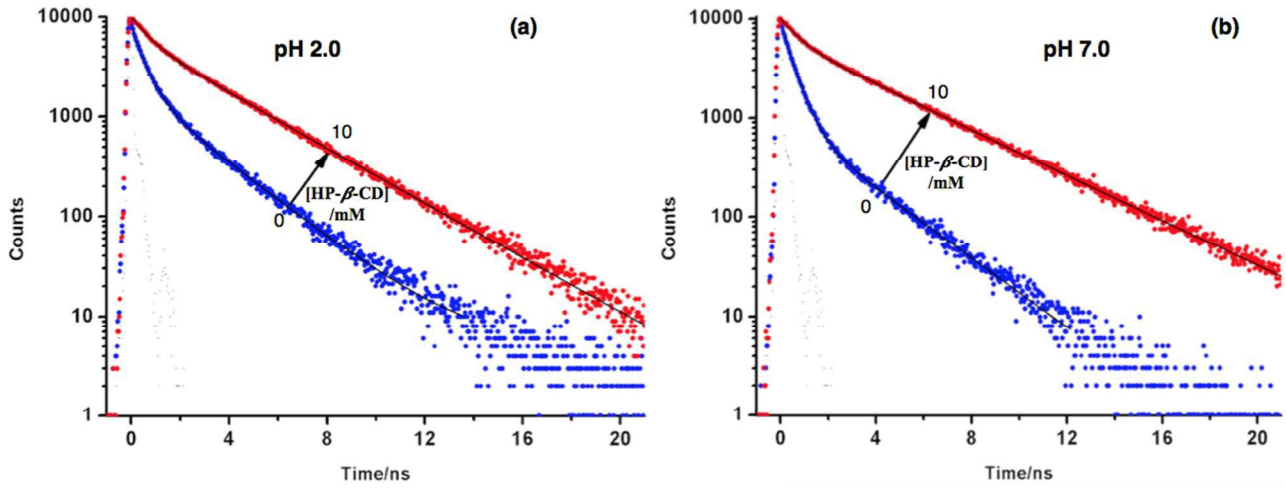

Figure 5. Effect of HP-*β*-CD encapsulation on fluorescence lifetime of DSS at (a) pH 2.0, (b) pH 7.0. Increase in fluorescence lifetime upon complexation using 10 mM HP-*β*-CD is shown by arrows.

As we have seen in steady-state measurements, at higher pH, in the case of neutral DSS, the binding strength is much stronger than that of lower pH and thus imparts greater influence on the fluorescence lifetime. Upon complexation with HP-*β*-CD the fluorescence lifetime at pH 2.0 enhances by 1.5 fold (from 2.0 ns to 3.1 ns, Fig. 5a) whereas in case of pH 7.0 it gets almost

doubled (from 1.9 ns to 3.7 ns, Fig. 5b). A summary of pH dependent lifetime values (Table 1) indicate a decrease in radiative decay rates of DSS upon HP-*β*-CD encapsulation. The time resolved fluorescence data strongly corroborates the results obtained from steady state studies.

3.5 Assaying binding strength of food-additives with HP-*β*-CD using displacement principle at pH 7.0

Polyphenolic compounds are natural antioxidant, receiving a constant attention in the areas of health and food processing industries.[42] The frequently used food-additives have an influential impact on human health. In fact, these additives are known to have multiple purposes such as taste enhancer, antioxidant activity, food preservation *etc.*. Due to the excessive consumption of food additives often creates a matter of health concern in terms of hormonal disorder, premature birth, indigestion, and ulcers in intestines, which ultimately cause cancers.[7] The limits of the use of the food additives become uncontrollable due to their poor water solubility, life span of existence within the physiological system. This is the high time to find out some way out of this present problem.[43] As a matter of fact, the CD based formulation of poor water soluble drugs is already popular and also commercialized.[44, 45] The non-toxicity, high water solubility and variable cavity size, and easy derivatization find CD as a well-suited macrocyclic molecule for encapsulation of food-additives for increasing solubility, stability, and control release for long-term action.[21, 46]

In this study, seven different non-fluorescent food-additives were selected *viz.*, monosodium glutamate, *trans*-ferulic acid, *p*-coumaric acid, gallic acid and their derivatives (for structure see Fig. 6a) to evaluate their binding with HP-*β*-CD as a macrocyclic receptor with a hydrophobic cavity. The supramolecular entity consists of DSS and HP-*β*-CD is a well-suited candidate for constructing a supramolecular displacement assay based on the enhanced fluorescence intensity of DSS upon relocation into HP-*β*-CD cavity as already reported for small drugs and antibiotics.[25] Weakly fluorescent or non-fluorescent molecules also having binding affinity with HP-*β*-CD can relocate highly fluorescent DSS from HP-*β*-CD cavity *via* competitive displacement. Interestingly, a titration of a preformed DSS•HP-*β*-CD complex with increasing concentration of competitor food additive molecules will result in reversal of fluorescence intensity, and this can allow us to determine the binding affinity of the competitor molecule. This competitive displacement strategy

for investigating the binding event of seven important food-additives *i.e.*, monosodium glutamate, *trans*-ferulic acid, *p*-coumaric acid, gallic acid, methyl gallate, ethyl gallate, and propyl gallate, with HP-*β*-CD has been implemented. A typical fluorescence displacement titration using DSS•HP-*β*-CD and Coumaric acid as a competitor is shown in figure 6a.

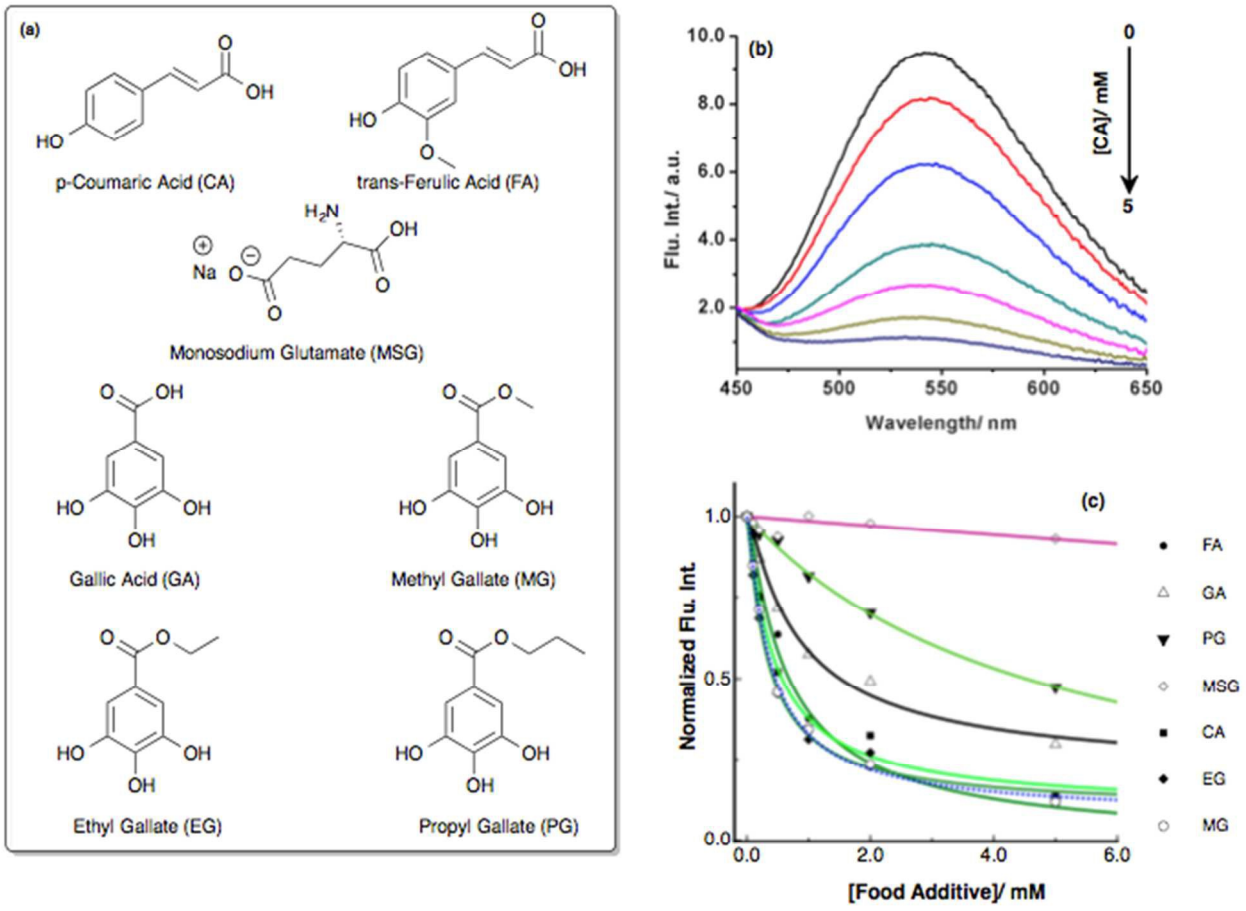

Figure 6. Binding study of food-additives with HP-*β*-CD using switch-off displacement assay (a) structure of studied food aditives; (b) Fluorescence spectra of DSS on addition of *p*-coumaric acid (CA) ranging concentration from 0-5 mM in a solution of 4 μM DSS and 5 mM HP-*β*-CD; (c) Change in fluorescence intensity of DSS at 550 nm on addition of FA-*trans*-ferulic acid, GA-gallic acid, CA-*p*-coumaric acid, MSG-monosodium glutamate, PG-propyl gallate, EG-ethyl gallate and MG-methyl gallate ranging concentration from 0-5 mM.

During displacement assay the pH of the solution of both complex and food-additives were adjusted to pH 7.4, and the concentration of DSS and HP-*β*-CD in the solution of food-additives were kept same to avoid the effect of change due to pH and dilution. Further, we have performed the fluorescence displacement assay in lower (pH 4.0) and higher (pH 9.0) and our result (figure S2) suggest similar binding trend and this suggest that the intermolecular interaction between HP-*β*-CD and the food-additives gets unaltered and ionic strength does not cause much effect on guest binding. Later the decrease in fluorescence intensity at fluorescence maxima against the concentration of competitor food additives is plotted and fitted with the previously published competitive binding equation.[47] The study unveils the fact that except monosodium glutamate (Binding constant K<20 M^{-1}) other food additives can be considered as potent candidate for formulation with HP-*β*-CD, as they have moderate binding affinity (*p*-coumaric acid K= 320 M^{-1}, *trans*-ferulic acid K= 555 M^{-1}, gallic acid K= 551 M^{-1}, methyl gallate K= 595 M^{-1}, ethyl gallate K= 380 M^{-1}, propyl gallate K= 250 M^{-1}) towards the cavity of HP-*β*-CD (Fig. 6b). Binding constant of the measured food additives are summarized in table 2. The binding constants of the investigated food-addtives with HP-*β*-CD is not significantly high and our study is a proof-of-concept on the application of fluorescence-based off assy for food-additive formulations.

Table 2: Binding constant of investigated food additive with HP-*β*-CD at pH 7.0

Food Additive	**FA**	**CA**	**MSG**	**GA**	**MG**	**EG**	**PG**
K/M^{-1}[b]	555	320	<20	551	595	380	250

[a] using competitive binding equation[47]

4. Conclusion

In this report, we have studied the effect of pH on supramolecular recognition of DSS and HP-*β*-CD. Host-guest complex formation between DSS and HP-*β*-CD has been characterized using UV-Vis. study and steady-state and time-resolved fluorescence spectroscopic techniques. In fact, the preferential binding affinity of different prototropic forms of DSS with HP-*β*-CD shifts acid-dissociation constant of encapsulated DSS and causes large (*ca*. 25 times) fluorescence enhancement. Such huge enhancement has been utilized to evaluate the binding affinity of seven

frequently used food-additives *via* switch-off displacement assay. Indeed, the demonstration of this displacement assay can be further utilized to evaluate the binding affinity of other frequently used non-fluorescent food-additives and small drug molecules. We also believe that the complex entity consists of DSS and HP-*β*-CD is a useful reporter system for pre-investigation of important small molecule prior to their real formulation.

SUPPORTING INFORMATION AVAILABLE

Electronic supporting information (ESI) is available containing fluorescence lifetime plot in other pH values from time-resolved fluorescence measurements.

ACKNOWLEDGMENT

We would like to thank Indian Institute of Science Education and Research Bhopal (IISER Bhopal) for providing the infrastructure and Department of Science and Technology, India (SERB/CS-253/2013) for research grant. KP thanks CSIR, SM thanks UGC and FC thanks IISERB for their doctoral fellowship.

REFERENCES

1. R. Laxminarayan, A. Duse, C. Wattal, A. K. M. Zaidi, H. F. L. Wertheim, N. Sumpradit, E. Vlieghe, G. L. Hara, I. M. Gould, H. Goossens, C. Greko, A. D. So, M. Bigdeli, G. Tomson, W. Woodhouse, E. Ombaka, A. Q. Peralta, F. N. Qamar, F. Mir, S. Kariuki, Z. A. Bhutta, A. Coates, R. Bergstrom, G. D. Wright, E. D. Brown and O. Cars, *Lancet Infect. Dis.*, 2013, **13**, 1057-1098.
2. U. Obolski, G. Y. Stein and L. Hadany, *PLoS Comp. Biol.*, 2015, **11**, e1004340.
3. D. McCann, A. Barrett, A. Cooper, D. Crumpler, L. Dalen, K. Grimshaw, E. Kitchin, K. Lok, L. Porteous, E. Prince, E. Sonuga-Barke, J. O. Warner and J. Stevenson, *Lancet*, **370**, 1560-1567.
4. C. Sullivan, S. S. Sayre, J. B. Leon and et al., *J. Am. Med. Assoc.*, 2009, **301**, 629-635.
5. G. D. Stoner, M. B. Shimkin, A. J. Kniazeff, J. H. Weisburger, E. K. Weisburger and G. B. Gori, *Cancer Res.*, 1973, **33**, 3069-3085.
6. F. A. Fairweather and C. A. Swann, *Proc. Nutr. Soc.*, 1981, **40**, 21-30.
7. K. L. Klump, *Horm. Behav.*, 2013, **64**, 399-410.
8. K. T. Savjani, A. K. Gajjar and J. K. Savjani, *ISRN Pharma.*, 2012, **2012**, 10.
9. J. K. Tobacman, R. B. Wallace and M. B. Zimmerman, *Med. Hypotheses*, 2001, **56**, 589-598.
10. E. F. Thomas, *CRC Handbook of Food Additives*, CRC press, Second Edition edn., 1972.

11. E. Lueck and M. Jager, *Antimicrobial Food Additives: Characteristics Uses Effects*, Springer, 2nd Ed. edn., 1995.
12. I. Volf, I. Ignat, M. Neamtu and V. Popa, *Chem. Papers*, 2014, **68**, 121-129.
13. A. P. Subramanian, A. A. John, M. V. Vellayappan, A. Balaji, S. K. Jaganathan, E. Supriyanto and M. Yusof, *RSC Adv.*, 2015, **5**, 35608-35621.
14. J. A. Vinson and Y. Cai, *Food Funct.*, 2012, **3**, 134-140.
15. X. Zhang, F. Chen and M. Wang, *J. Agric. Food Chem.*, 2014, **62**, 1643-1648.
16. B. Badhani, N. Sharma and R. Kakkar, *RSC Adv.*, 2015, **5**, 27540-27557.
17. C.-L. J. Teng, S.-M. Han, W.-C. Wu, C.-M. Hsueh, J.-R. Tsai, W.-L. Hwang and S.-L. Hsu, *Food Chem. Toxicol.*, 2014, **71**, 197-206.
18. S. Nandi, M. Vracko and M. C. Bagchi, *Chem. Biol. Drug Des.*, 2007, **70**, 424-436.
19. C. D. Selassie, S. Kapur, R. P. Verma and M. Rosario, *J. Med. Chem.*, 2005, **48**, 7234-7242.
20. I. Ghosh and W. M. Nau, *Adv. Drug Deliv. Rev.*, 2012, **64**, 764-783.
21. M. E. Brewster and T. Loftsson, *Adv. Drug Deliv. Rev.*, 2007, **59**, 645-666.
22. A. Munin and F. Edwards-Lévy, *Pharmaceutics*, 2011, **3**, 793.
23. N. Saleh, A. L. Koner and W. M. Nau, *Angew. Chem. Int. Ed.*, 2008, **47**, 5398-5401.
24. A. L. Koner, I. Ghosh, N. Saleh and W. M. Nau, *Can. J. Chem.*, 2011, **89**, 139-147.
25. K. Pal, S. Mallick and A. L. Koner, *Phys. Chem. Chem. Phys.*, 2015, **17**, 16015-16022.
26. R. N. Dsouza, U. Pischel and W. M. Nau, *Chem. Rev.*, 2011, **111**, 7941-7980.
27. K. Uekama, F. Hirayama and T. Irie, *Chem. Rev.*, 1998, **98**, 2045-2076.
28. M. Sayed and H. Pal, *Phys. Chem. Chem. Phys.*, 2015, **17**, 9519-9532.
29. J. Szejtli, *Chem. Rev.*, 1998, **98**, 1743-1754.
30. W. M. Nau, M. Florea and K. I. Assaf, *Isr. J. Chem.*, 2011, **51**, 559-577.
31. A. L. Koner and W. M. Nau, *Supramol. Chem.*, 2007, **19**, 55-66.
32. Z. J. Diwu, C. L. Zhang, D. H. Klaubert and R. P. Haugland, *J. Photochem. Photobiol. A - Chem.*, 2000, **131**, 95-100.
33. S. Mallick, K. Pal and A. L. Koner, *J Colloid Interf Sci*, 2016, **467**, 81-89.
34. K. Pal, F. Chandra, S. Mallick and A. L. Koner, *J. Photochem. Photobiol. A: Chem.*, 2015, **306**, 47-54.
35. X. Y. Zhang, G. Gramlich, X. J. Wang and W. M. Nau, *J. Am. Chem. Soc.*, 2002, **124**, 254-263.
36. H. Bakirci, A. L. Koner and W. M. Nau, *Chem. Commun.*, 2005, 5411-5413.
37. Z. R. Grabowski, K. Rotkiewicz and W. Rettig, *Chem. Rev.*, 2003, **103**, 3899-4031.
38. C. Marquez and W. M. Nau, *Angew. Chem. Int. Ed.*, 2001, **40**, 3155-3160.
39. J. Mohanty, A. C. Bhasikuttan, W. M. Nau and H. Pal, *J. Phys. Chem. B*, 2006, **110**, 5132-5138.
40. H. Bakirci, A. L. Koner, T. Schwarzlose and W. M. Nau, *Chem. -Eur. J.*, 2006, **12**, 4799-4807.
41. M. Shaikh, J. Mohanty, P. K. Singh, W. M. Nau and H. Pal, *Photoch Photobio Sci*, 2008, **7**, 408-414.
42. R. Amorati and L. Valgimigli, *Org. Biomol. Chem.*, 2012, **10**, 4147-4158.
43. R. Walker, E. A. Decker and D. J. McClements, *Food Funct.*, 2015, **6**, 41-54.
44. V. Stella and R. Rajewski, *Pharm. Res.*, 1997, **14**, 556-567.
45. M. E. Davis and M. E. Brewster, *Nat. Rev. Drug. Discov.*, 2004, **3**, 1023-1035.
46. E. A. Nazli and B. Erem, in *Nanotechnology and Drug Delivery, Volume One*, CRC Press, 2014, vol. 1, pp. 178-209.

47. H. Bakirci and W. M. Nau, *Adv. Funct. Mat.*, 2006, **16**, 237-242.

TOC Graphic: