Host–guest interactions between cucurbit[7]uril (CB7) and a cardio-tonic drug, milrinone, have been explored using steady state and pico-second time-resolved techniques. A novel fluorescence switch from ultraviolet (UV) to visible (cyan) is observed as a consequence of upward $pK_a$ shift of the drug inside the nano-cavity of cucurbit[7]uril.

Recently, host–guest chemistry has attracted considerable interest owing to its versatile applications in the delivery, stabilization, solubilization and controlled release of drugs as well as analyte sensing. Among the vast repertoire of classical macrocyclic host molecules, like, cyclodextrins, calixarenes, crown ethers, etc., the cucurbiturils, e.g., cucurbit[7]uril (Fig. 1A) in particular, recently, have emerged as versatile hosts due to their remarkable guest-binding behavior. The pumpkin shaped macrocycles composed of $n$ glycoluril units can accommodate neutral molecules through hydrophobic interactions, whereas the carbonyl portals can form complexes with cations through ion-dipole and/or hydrogen bonding interactions. These versatile properties caused CB$n$ to have widespread applications, such as, reaction containers, molecular necklaces, on–off switches, porous materials, supra-molecular tandem enzyme assays and control of aggregate formation. Recently, it has been established that CB7 can cross the cell membrane and the host has a low toxic effect on the cell. Since then the study of inclusion complexes of several drug molecules with CB7 has been the subject of interest. Very recently, we have investigated the inclusion complexes of several antitumor drugs with CB7 using steady state, time-resolved and other spectroscopic techniques. In continuation of our effort, herein, we have documented the inclusion complexation study of a heart medicine, milrinone with CB7. Milrinone (1,6-dihydro-2-methyl-6-oxo-3,4$^\text{0}$-bipyridine-5-carbonitrile) is a synthetic cardiotonic medicine widely used in the treatment of congestive heart failure. The mechanism of the action of this drug is through the elevation of 3$^\text{0}$,5$^\text{0}$-cyclic adenosine monophosphate (cAMP) levels in the myocardial cell by inhibiting the cyclic nucleotide phosphodiesterase. Though milrinone (MIR) exists in different forms (keto, enol, cation and anion) depending upon the pH of the medium, only cationic and keto forms (Fig. 1) are known to be medicinally active. The drug has limitations in oral usage due to its adverse side effects, like thrombocytopenia, fever, anorexia, abdominal pain, nausea and emesis. The formulation of the drug with less toxic materials may reduce some of the side effects. As CB7 acts as a non-toxic drug delivery vehicle, we thought the interaction behavior between the drug and CB7 might provide a new insight into the delivery aspect of the drug. Astonishingly, a fluorescence switch from ultraviolet (UV) to visible (cyan) is observed, when the drug is encapsulated within the CB7 nano-cavity. We also noticed that the biologically active cationic form ([MIR]$^+$) of the drug selectively exists within the CB7 nano-cavity. Interestingly, cyan color of the drug in the presence of CB7 switches back to violet upon increasing the pH of the medium (Fig. 2). We hope that this kind of UV to visible color switch can be utilized to probe the drug in the delivery process.
Earlier report confirmed that the emission at ~475 nm under acidic conditions arises due to the cationic form of the drug.\textsuperscript{22} Therefore, we reckon that the peak at 500 nm, which is observed when MIR binds with CB7, might be attributed to the cation form (MIRH\textsuperscript{+}) of the drug. This infers that the pK\textsubscript{a} value for the conversion of keto to cation form of the drug gets affected when MIR binds with CB7. It is well known that CB7 is prone to shift the pK\textsubscript{a} value upward for many biological drugs.\textsuperscript{14,16,17} To prove whether the increased pK\textsubscript{a} value is the cause for the appearance of cyan color in the presence of CB7 or not, we have further measured pK\textsubscript{a} of MIR in the presence of CB7, and we found that the pK\textsubscript{a} value of MIR is increased to 7.1 in the presence of 500 µM of CB7. Moreover, we have also noticed that the cyan color of MIR in the presence of CB7 switches back to violet in the solution of pH above 7.1. To ensure the formation of cationic MIR (MIRH\textsuperscript{+}) in the presence of CB7, we have performed similar experiments at acidic pH (pH 4) and basic pH (pH 10). It is visible from Fig. S1 (ESI\textsuperscript{†}) that the interaction pattern at pH 4 is quite similar to that of neutral aqueous solution. Whereas at pH 10, the drug does not exhibit any interaction with CB7, as MIR exists in anionic form at this pH, and anionic species generally does not interact with CB7 due to the presence of electron rich carbonyl portals. All these observations lead us to conclude that there is a pronounced pK\textsubscript{a} shift from 4.5\textsuperscript{22} to 7.1, when MIR binds to CB7. It is well known that CB7 can increase the pK\textsubscript{a} values of many drugs due to its high electron density carbonyl portals.\textsuperscript{14,16,17} Therefore, we believe that the observed upward pK\textsubscript{a} shift is attributed to the residence of pyridyl nitrogen near the electron rich carbonyl portals of CB7, and this will be further verified through docking followed by quantum chemical calculations. The stoichiometry and binding affinity are calculated by using the Benesi-Hildebrand (BH) plot (Note S1, ESI\textsuperscript{†}). Linear BH analysis (Fig. S2, ESI\textsuperscript{†}) confirms the 1:1 complexation between CB7 and MIR with a binding constant of \(3.7 \times 10^4\) M\textsuperscript{-1}. The 1:1 stoichiometry is further confirmed from Job’s method of continuous variation (Fig. S3, ESI\textsuperscript{†}). The proposed multiple equilibria of complexations between the drug and CB7 at different pH are shown in Scheme 1. To see the effect of pH on binding affinity of MIR with CB7, we have also calculated binding constants at pH 4 from the titration curve (Fig. S4, ESI\textsuperscript{†}) using the BH plot. The binding constant calculated at pH = 4 is found to be \(4.0 \times 10^4\) M\textsuperscript{-1}, which is slightly higher compared to the value obtained under neutral conditions. This is expected because at this acidic pH, cationic species predominantly exist in the solution, and thereby, they are easily attracted by the electron rich carbonyl portals of CB7. At pH 10, as the interaction between anionic form of the drug and CB7 is almost negligible (Fig. S1b, ESI\textsuperscript{†}), we were not able to determine the binding constant.

\[
\text{MIR} + \text{CB7} + \text{H}_2\text{O} \rightarrow \text{MIRH}^+ + \text{CB7} + \text{H}_2\text{O} \quad (\text{In acidic solution})
\]

\[
\text{MIR} + \text{CB7} + \text{H}_2\text{O} \rightarrow \text{MIR}^+ \cdot \text{CB7} + \text{OH}^- \quad (\text{In neutral solution})
\]

**Scheme 1** Representation of multiple equilibria of MIR and CB7 complexation.
To explore the dynamics of MIR within CB7, we have employed picosecond time-resolved measurements. The fluorescence transients of MIR in aqueous solutions and in the presence of CB7 are shown in Fig. 3A and Fig. S5 (ESI†), and the fitting results are summarized in Table S1 (ESI†). In water, MIR exhibits double exponential decay (collected at 390 nm) with time constants of 90 ps and 2.2 ns. The former component is believed to have originated from keto form, whereas the latter component is attributed to the relaxed state of keto due to the ICT character.22 In the presence of CB7, the decays collected at 500 nm (cation form) exhibit a tri-exponential feature with two nanosecond components and a picosecond component (Table S1, ESI† and Fig. 3A). Similar lifetime components of MIR were also observed in the presence of a PMMA rigid matrix, and were attributed to the restriction of free rotation of a pyridine moiety, which is the major non-radiative decay channel of MIR.23 Therefore, we reckon the nanosecond components appeared in the decay profile is an outcome of the inhibition of free rotation of the drug inside the nano-cavity. The decay profile of MIR at 390 nm also consists of nano-second components (3.14 ns to 5.43 ns), and it is attributed to CB7 bound cationic species of the drug, as the emission of cationic species also contributes towards 390 nm. To prove the inclusion complexation between CB7 and MIR, we have employed rotational relaxation measurement of the drug in the absence and presence of CB7 (Fig. 3B) with the help of time-resolved anisotropy measurements. The anisotropy decay of MIR in aqueous solution exhibits a mono-exponential feature in nature with a rotational relaxation constant of 150 ps. A bi-exponential decay behavior is observed in anisotropy transient of MIR in the presence of 500 μM of CB7 with time constants of 90 ps and 680 ps. Here 90 ps observed for MIR in aqueous solution can be assigned as unbound or free MIR and 680 ps is due to MIR bound with CB7. The increase in rotational relaxation time (τr) from 150 ps to 680 ps in the presence of CB7 confirms the formation of inclusion complexes where the drug experiences a more rigid environment inside the nano-cavity. This τr value is used to calculate the hydrodynamic radius of the inclusion complex according to the Stokes–Einstein relationship (Note S2, ESI†). The calculated hydrodynamic diameter value, 17.6 Å, is in good agreement with that of the 1:1 inclusion complex between CB7 and MIR.

Furthermore, docking and semi empirical methods (PM3MM) are employed to obtain the optimized structure of the CB7:MIRH+ inclusion complex. As shown in Fig. 4 the drug is completely buried inside the CB7 nano-cavity. It can be clearly seen from the optimized structure that the pyridine nitrogen resides very close to the electron rich carbonyl portals of CB7, and thereby it experiences high electron density in the inclusion complex. As a result, the pK_a value of pyridine nitrogen increases from 4.5 to ~7.1. Therefore, theoretical studies well corroborate the steady state and time-resolved experimental observations.

Conclusions

In summary, the interaction behavior between a heart medicine, milrinone, and cucurbit[7]uril has been demonstrated with the help of absorption, steady state and time-resolved emission measurements. Amazingly, the fluorescence of MIR switches from UV to visible (cyan color) as a result of complexation (Scheme 1), and we attribute this fluorescence switch to the increased pK_a of protonation of pyridine nitrogen. However, visible fluorescence (cyan color) switches back to UV with increasing pH of the solution. The molecular picture obtained from docking and quantum chemical calculation depicts that pyridine nitrogen of MIR is situated at the electron rich portal of CB7 in the inclusion complex, and as a result the basicity of pyridine nitrogen increases significantly. We believe that this kind of
fluorescence switch is rarely observed with drug molecules, and therefore, it can be exploited to monitor the delivery process of the drug.

Acknowledgements

This work was partly supported by the Council of Scientific and Industrial Research (CSIR), Government of India. AS is thankful to CSIR for the Senior Research Fellowship and RKK is thankful to University Grants Commission (UGC) for the Junior Research Fellowship. The authors thank the Director, IISER-Pune, for providing excellent experimental and computation facilities. The authors are thankful to anonymous reviewers for their valuable comments and suggestions.

Notes and references