pH-induced reversible molecular self-assembly of perylene based imidazolate ester: a probable marker for biological species

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Dianhydrides of perylene-3,4,9,10-tetracarboxylic acid (PTCDA) provide an excellent backbone for design and synthesis of various molecules of interest. Many water-soluble perylene derivatives with stimuli responded functionality being reported but there is few pH responded perylene derivatives were synthesized. The PTCDA derivatives so far synthesized are pH sensitive. These derivatives in an acid medium exist in its monomer form and undergo self-assembly with an increase in pH. In this paper, we report synthesis of pH-sensitive perylene based imidazolate ester, which exists in monomer form at pH 12 and undergo self-assembly with the decrease in pH contrary to the earlier reports. This phenomenon can be well explained for a probable marker in biological processes occurring specifically at pH 12.

Keywords: 2-(3,5-Dimethoxy-phenyl)-4,5-dimethyl-imidazol-1-ol, Hydrophobicity, PTCDA

Many biological reactions are stimulated by various physical parameters such as temperature¹, pressure², pH³ and external ions. Various smart materials are being developed from time to time to monitor such biological changes. Supramolecular structures (formed by the self-assembly of monomeric units) having multifunctional groups are susceptible to such kind of parameters and thus, become good candidates to monitor such biological changes. The interactions between the monomeric units are mostly with intermolecular and/or π-π interaction⁴ and hydrogen bonding⁵. While other noncovalent interactions such as hydrogen bonding are regarded as a major driving force for promoting self-assembly in solution, π-stacking, is usually considered as a secondary motif⁶. Further, the intramolecular interaction leads to folding, and intermolecular interaction is responsible for self assembly⁷. The probability of formation of π-π stacking increases with the use of the nonpolar solvent medium, whereas in polar solvents the self-assembly is attributed to hydrophobicity and/or π-π stacking.

Dianhydrides of perylene-3,4,9,10-tetracarboxylic acid (PTCDA) provide an excellent backbone for design and synthesis of various molecules of interest either by functionalizing the anhydride through imide formation or by making an appropriate substitution at the bay position available. These two positions have been explored successfully and a large number of perylene based derivatives have been synthesized and their application in organic field effect transistors⁸-⁹, OLEDs¹⁰-¹¹, Organic Solar cell¹²-¹³ has been studied. It is known that perylene based dyes form a π-stacked structure in crystals and get self-organized in a co-facial configuration adopting herringbone packing or slipped co-facial configuration¹⁴.

Based on the mechanism of operation, pH indicator dyes are classified into absorption and fluorescent¹⁵. Phenol red, bromocresol green or phenolphthalein are examples of absorption based pH indicators¹⁶. The optical properties of the dyes change with the modification of pH of the surrounding environment. In the same time, the change in colour of the sensing membrane in which the pH indicator is incorporated can be detected. In contrast to the absorption based dyes, fluorescent based indicators have better selectivity and stability¹⁷, but usually suffer from optical changes regarding emission spectra variation and fluorescence intensity¹⁸. Some fluorescent pH sensors have already been established in which derivatives of 8-hydroxyppyrene-1,3,6-trisulfonate¹⁹, fluoresceins²⁰, and benzo[g]xanthene dyes²¹, 1,8-naphthalimide²² have been the most common pH-sensitive indicator dyes.
Many water-soluble perylene derivatives with stimuli responded functionality being reported but there is few pH responded perylene derivatives were synthesized. Since pH is a critical stimulus thus, it is thought worthy to synthesize perylene derivatives with excellent pH response. Water-soluble perylene derivatives employed for pH sensing have been reported by Gan et al.\textsuperscript{23} and Xu et al.\textsuperscript{24}. D-\pi-A framework with indole as donor and benzothiazole as an acceptor dye have been designed and synthesized by Gwan et al.\textsuperscript{25}, which provide an optical switching in the presence of alkali/acid. The prepared dye shows clear fluorescence quenching with the addition of alkali. Synthesis of water-soluble amino-imidazole armed perylene diimide dye for biological application has been reported\textsuperscript{26} and employed for detection of glucose. Based upon the molecular self-organization of perylene based derivatives, we report the tuning of optical emission of pH-induced molecular self-assembly in aqueous solution. On changing the pH from 12 to 7, it shows a change in colour from green to orange and finally red. Although, many perylene derivatives are reported to exhibit such pH based self-assembly while moving from low pH to high pH, self-assembly of perylene derivative by lowering of pH has not been reported so far.

Materials and Method

All the chemicals are procured from Sigma-Aldrich India Ltd. and used as such. All the solvents are procured from Merck and used without further purification. UV-Vis spectral data were recorded in Shimadzu-UV3600 plus. IR spectra were recorded in Perkin-Elmer IR Spectrophotometer. Fluorescence spectra were recorded in Horiba Scientific Fluoromax-4. 2,4,5-trisubstituted-1-hydroxy imidazoles can be synthesized convincingly employing reported procedure\textsuperscript{27}. These synthetic imidazoles on reaction with PTCDA in 1:1 ratio in acetone and potassium carbonate gave convincingly the desired product I (Scheme-1: Synthesis of I).

2-(3,5-Dimethoxy-phenyl)-4,5-dimethyl-imidazol-1-ol (1 mM, 0.248 g) and K\textsubscript{2}CO\textsubscript{3} (2 mM, 0.276 g) were dissolved in acetone and stirred magnetically for 30 min at room temperature then PTCDA (1 mM, 0.392 g) was added to the reaction mixture and stirred for 12 h. Water was added to the reaction mixture, and precipitate thus, obtained was filtered off, washed with distilled water several times and then dried.

All the synthetic compounds are characterized by IR and UV-Vis spectra. Attempt to obtain NMR spectra went in vain as compound I aggregate in the NMR tube due to the solubility problem. The spectral data of 2,4,5-trisubstituted-1-hydroxy imidazoles are in agreement with the reported data\textsuperscript{28}.

Result and Discussion

The UV-VIS absorption spectroscopy shows (Fig. 1A) three absorption bands A\textsubscript{0-0} (467 nm), A\textsubscript{0-1} (437 nm), A\textsubscript{0-2} (411 nm), respectively. The intensity ratio between A\textsubscript{0-0} and A\textsubscript{0-1} is 0.35 smaller than 1.6, indicating a less dominated aggregation state of the molecule at pH 12. With the increasing concentration the ratio between A\textsubscript{0-0} to A\textsubscript{0-1} decreases showing the commencement of aggregation at the concentration of 5 × 10\textsuperscript{-5} M. With the addition of acid, the aggregation is more marked even at the same concentration. Fluorescence measurements were further used to examine the pH-induced aggregation. It shows the characteristic peaks at 490 and 511 nm when excited at 438 nm in phosphate buffer medium.

The fluorescence intensity decreases sharply as we move from pH 12 to 11 (Fig. 1B) and after that gradually decrease with a decrease in pH and finally disappears below 9. The plot of logarithmic of fluorescence intensity vs. pH (Fig. 1C) exhibits a linear relationship suggesting switching of the monomeric state with the aggregation state due to intramolecular fluorescence energy transfer\textsuperscript{29}. When excited at 490 nm, emission occurs at 590 nm (Fig. 2 A & B), and the pH dependence is well marked as it imparts an orange fluorescence in lower pH with aggregation and a green fluorescence at pH 12 (Fig. 3). The peak at 590 nm totally vanishes below pH 5 showing complete aggregation.

The loss of anhydride peak (1772 cm\textsuperscript{-1}), and formation of esteric (1740 cm\textsuperscript{-1}) and carboxylate peak
(1575 cm$^{-1}$) in the IR spectra justifies the formation of the esteric product. Further, the anhydride peak at 1772 cm$^{-1}$ reduces its intensity and a new peak at 1740 cm$^{-1}$ develops suggesting the formation of monesteric product. In acid medium; the peak at 2926 and 2857 cm$^{-1}$ becomes sharp showing the free C-H stretching of the methyl group.

Zhang et al.$^{26}$ have proposed the existence of monomer in acidic condition and aggregation of these monomers at pH 7. As reported, it exhibits orange fluorescence in acidic medium and on aggregation it forms long hybranched nanobelts at pH 8 as indicated by the TEM image. Similarly, Wang et al.$^{29}$ have reported formation of rainbow colour photoluminescence in CHCl$_3$.

The SEM image of the compound at pH 7 (Fig. 4) shows the self-assembly is due to hydrophobicity and at this moment we have not further explored to calculate the hydrophobicity factor. Fibre-like association of molecules has been observed when magnified probably due to the intermolecular hydrogen bonding. However, the SEM image at pH 12 (Fig. 5), shows different patterns and scattered structures clearly suggesting the existence of monomeric unit. The cubical structure has been attributed as the molecule is existing in its sodium salt form at pH 12.

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Fig. 1—UV-Vis absorbance spectra of Compound I at concentration $1-9 \times 10^{-5}$ M PIE at pH 12 (A); linear relationship between Ln (FL) and pH (B); and Fluorescence emission spectra of $1-50 \times 10^{-5}$ M of Compound I in the buffer at pH 12 (C)

Fig. 2—Absorption (A); and emission (B) spectra of Compound I at pH 3-12 in buffer medium

Fig. 3—Colour change with pH as observed in naked eye (A); and PIE in buffer at low pH (3, left) and high pH (12, right) (B)
Thus, we propose that the equilibrium exists between the carboxylic acid and its sodium salt in acid and alkaline medium respectively as shown in Fig. 6. The carboxylic group present in the molecule undergoes protonation/deprotonation controlling the solubility of the molecule. In acid medium the molecule is coagulating may be due to the aggregation caused by intermolecular hydrogen bond formation as shown below. This may result in the loss of planarity, which, leads to the reduction in the rigidity of the system thereby favoring the internal conversion process. In an alkaline medium, it forms the corresponding carboxylate ion which does not have acidic hydrogen to exhibit necessary hydrogen bond (Fig. 6).

Conclusion

Synthesis of the perylene based imidazolate ester has been achieved by reacting PTCDA with 2-(3,5-
Dimethoxy-phenyl)-4,5-dimethyl-imidazol-1-ol. The imidazolate ester thus obtained exhibits pH dependence. Contrary to the earlier reported pH sensitive perylene based derivatives, we report for the first time the existence of the aggregated state in acid medium and monomeric form in alkaline medium. As the pH of cancer cells is lower than the normal cells and the extracellular environment of these cells are more acidic than the normal cells. Hence, the molecule in hand can be applied as a potential contender for the diagnosis of cancer cells by making it alkaline.

References