Novel excited state proton transfer reaction observed for proflavine encapsulated in the channels of modified MCM-41

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Abstract

Proflavine (3,6-diamino acridine) was encapsulated in mesoporous MCM-41 and in a silane modified MCM-41. Synthesised MCM-41 and modified MCM-41 materials were characterised by XRD, BET, and FT-IR methods. In the dye-encapsulated mesoporous materials the interactions between encapsulated proflavine and the internal surfaces of the porous materials are found to modify the optical spectra and the excited state dynamics of the confined proflavine molecules. In MCM-41 as evidenced by the absorption and emission spectra, proflavine exists in the monocationic form (PFH⁺) with a characteristic absorption maximum at 444 nm. In the silane modified MCM-41, proflavine is found to be present in the neutral form with the characteristic absorption maximum at 394 nm. Steady state fluorescence spectra for all the dye-encapsulated mesoporous materials and lifetimes of the excited state of proflavine encapsulated in these hosts were investigated. Excited state proton transfer reaction is observed for the dye lodged in the nanometer-sized pores of the silane modified MCM-41. A delayed emission in the picosecond time resolved emission decay measurements of the excited state of the dye confirms this process.

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1. Introduction

Excited state processes of chromophores in host materials find novel applications in optical storage systems. Applications towards artificial photosynthetic mimics and potential use of these compounds as sensor materials and in self-organising systems are well documented [1–4]. Chromophores encapsulated in inorganic molecular sieves show wide range of novel transformation of organic molecules. Synthesis of mesoporous MCM-41 [5] silicates and related materials leads to novel functional materials in the field of host–guest chemistry when suitable guests with appropriate hosts are designed. These novel materials have been used extensively in catalysis [6], polymerisation process [7], separation processes [8,9], preparation of nanoparticles [10] and photochemistry [11].

The synthesis and properties of ordered mesostructured and mesoporous inorganic based materials are currently drawing widespread attention and a variety of aligned species have been encapsulated in MCM-41 mesoporous molecular sieve. The photophysical and photochemical behaviour of organic dyes encapsulated in mesoporous materials are widely studied. Photosensitization of semiconductor nanoparticles in the micro and mesoporous materials leads to effective charge separation [12–14]. The structural characteristics of these designed materials enable the incorporation of optically active guest molecules in (a) crystallographically defined positions and (b) highly organised arrangements [15]. MCM-41 is a versatile host owing to its high thermal stability, high surface area, high pore volume and very narrow pore size distribution tunable in the range of 20–100 Å. A structural model for MCM-41
consists of a hexagonal arrangement of cylindrical pores embedded in a matrix of amorphous silica.

Electronic absorption and emission spectral studies of molecular-sieve-encapsulated chromophores have been of immense value to understand the influence of the environment on the electronic states of the guest molecules. Encapsulation of organic molecules in molecular sieves leads to changes in their optical, physical and chemical properties [16]. Spectroscopic studies of rhodamine B doped composites were studied by Hoppe et al. [17]. Yamashita et al. reported that the lifetime of rhodamine B in MCM-41 was longer than that observed on silica gel or in ZSM-5 [18]. Photoluminescence spectral properties of coumarin 540 doped in MCM-41 were studied by Gu et al. [19], which compared the photophysical properties of the dye in MCM-41 with those in ethanol solution. Photophysical studies on tris(2,2′-bipyridine) ruthenium(II) complex loaded in mesoporous silica were carried out by Ogawa et al. [20]. A rare earth complex was recently encapsulated in mesoporous silicates which showed that the photophysical properties are sensitive to the local environment [21]. Aggregation of the thionine dye within AlMCM-48 was also reported earlier [22]. Investigation on the spectroscopy and dynamics of a laser dye 4-(dicyanomethylene)-2-methyl-6-(p-dimethylaninostyril)-4H-pyran encapsulated in MCM-41 mesoporous material and Zeolite-Y was reported [23]. Photophysical properties of some coumarin derivatives incorporated in MCM-41 were studied by Li et al. recently [24].

Proflavine, an acridine class of dye is used largely in photobiology and photochemistry. The dye was used as a probe to understand the specific binding site at the polynucleotides in biological systems from the behaviour of the fluorescence properties on the binding sites [25]. Proflavine is also used as a sensitisers in light-induced hydrogen evolution from water, a system of interest in the photochemical storage of solar energy [26,27]. Acid–base equilibria involving the proflavine singlet state in aqueous solution and micellar solutions [28] were also studied. The adsorption characteristics and spectroscopic properties of proflavine in various heterogeneous assemblies such as clays, cellulose and zeolites were studied [30–32]. In this report, we have investigated the influence of the microheterogeneous environment provided by mesoporous materials on the absorption and emission spectral properties of proflavine molecules encapsulated in MCM-41 and modified MCM-41. Here in we have also shown how a fluorescent organic molecule is useful in sensing the acid–base properties of the microenvironment from the spectral studies.

2. Experimental methods

2.1. Chemicals

Proflavine hemisulfate (3,6-diamino acridine) was obtained from Fluka (Switzerland) chemicals. Cetyltrimethylammoniumbromide (CTAB) and tetraethylorthosilicate (TEOS) were obtained from Lancaster. All other chemicals were purchased from the Qualigens and Merck fine chemicals. Standard methods are followed for the purification of the common organic solvents. All other chemicals were of analytical grade and are used as received unless otherwise stated. Water used in this investigation was triply distilled over alkaline potassium permanganate in an all glass apparatus and was used for all the experiments.

2.2. Synthesis of MCM-41 and modified MCM-41

MCM-41 was synthesised by following the procedure already reported in the literature [33]. n-Cetyltrimethylammoniumbromide (2.4 g), CTAB was dissolved in 120 g of deionised water and stirred until the solution was homogeneous and clear. To this mixture 8 mL of ammonium hydroxide was added and stirred for 5 min after which 10 mL of tetraethylorthosilicate was added to give a molar composition of the gel (1 M TEOS:1.64 M NH₄OH:0.15 M CTAB:126 M H₂O). The reaction mixture was stirred overnight, after which the solution was filtered and washed consecutively with deionised water and ethanol; calcination was performed at 823 K for 5 h.

The modification of the interior structure of MCM-41 was carried out through the use of a silylation reagent 3-aminopropyltriethoxysilane, APTES. The silane modified MCM-41 (SMCM-41) was prepared according to a procedure detailed [34]. About 0.6 g of the calcined MCM-41 was mixed in a chloroform solution of 3-APTES (50 mL, 0.15 M) and stirred overnight at room temperature. The precipitate was filtered and washed with chloroform and dichloromethane.

2.3. Encapsulation of proflavine in mesoporous hosts by impregnation method

Proflavine with MCM-41 or SMCM-41 composites were prepared by stirring the aqueous solution of the dye and the MCM-41 or SMCM-41 for 6–8 h at room temperature which results in the loading of proflavine in mesoporous hosts. The material was filtered, washed thoroughly until the filtrate shows no absorbance at 445 nm. The amount of the dye loaded in mesoporous hosts was adjusted by taking different amounts of dye in aqueous solution.

2.4. Instrumentation

X-ray powder diffraction data of the MCM-41 and SMCM-41 materials were collected on a Pananalytical X’pert Pro X-ray diffractometer. XRD patterns were obtained between 0.5 and 10°. Adsorption and desorption isotherms were measured at 77 K on a Micromeritics ASAP 2010 instrument using nitrogen gas. The samples were outgassed at 423 K and 1 mPa for 14 h before adsorption measurements. The specific surface area of these mes-
oporous materials was determined by the standard BET method. The infrared spectra of the samples were recorded in a Shimadzu Model-8300 FT-IR spectrophotometer in the range 4000–400 cm\(^{-1}\) using KBr pellets.

UV–vis diffuse reflectance spectral studies of the samples were recorded using Agilent 8453 diode array spectrophotometer equipped with labsphere RSA-HP-8453 reflectance accessory. Steady state fluorescence measurements for the solid opaque samples were carried out with Hitachi F-4500 fluorescence spectrophotometer at the front face configuration at 45°. Perkin–Elmer MPF-44B fluorescence spectrophotometer interfaced with PC through RISH-COM-100 multimeter was also used to record fluorescence spectra.

Time resolved fluorescence decays were obtained by the time correlated single photon counting (TCSPC) method. A diode pumped Millenna V CW laser (Spectra Physics, 532 nm) was used to pump the Ti–sapphire rod in Tsunami picosecond mode locked laser system (Spectra Physics). The 750 nm (85 MHz) beam was taken from the Ti–sapphire laser and passed through pulse picker (Spectra Physics, 3980 2S) to generate 4 MHz pulses. The second harmonic output (375 nm) was generated by a flexible harmonic generator (Spectra Physics, GWU 23PS). The horizontally polarized 375 nm laser was used to excite the sample. The fluorescence emission at magic angle (54.7°) was counted by a MCP PMT (Hamamatsu R 3809U) after passing through the monochromator and processed through CFD, TAC and MCA. The instrument response function for this system is \(\sim 52\) ps. Fluorescence decay was measured at the front face configuration with suitable cutoff filters to avoid scattered light and the decay curve was analysed using IBH (UK) software (DAS-6).

3. Results and discussion

Powder X-ray diffraction measurements reveal that the MCM-41 synthesised presents a well-ordered lattice and Fig. 1a characterises the powder X-ray diffractogram at low angles. The XRD patterns of dye-encapsulated mesoporous hosts shown in Fig. 1b were also quite similar to that of the MCM-41. Since there was not much difference in the intensity of the XRD patterns among these samples, there seems to be no significant collapse of the hexagonally packed array structure during the encapsulation process and the MCM-41 structure is retained after proflavine is dispersed in its mesoporous channels. Powder X-ray diffractogram of SMCM-41 is presented in Fig. 1c. The XRD pattern of SMCM-41 indicates strong (100) peak, suggesting that framework stability is maintained for silane modified MCM-41. The BET surface area of 933.4 m\(^2\)/g was obtained for calcined MCM-41 from the isotherm, which shows a decrease in the surface area after the encapsulation of proflavine dye molecules in the pores of MCM-41. The BET surface area obtained for proflavine loaded MCM-41 is 791 m\(^2\)/g (Table 1), which shows the encapsulation of the proflavine molecule in the mesopores of MCM-41.

The IR spectra of these mesoporous molecular sieves show a series of bands that are characteristic of the SiO\(_4\) tetrahedral unit. The spectra show two main absorption bands between the regions 1245–1210 cm\(^{-1}\) and 1090–1055 cm\(^{-1}\) (supporting material). These bands are assigned to the asymmetric-stretching mode of SiO\(_4\) tetrahedral units in the silicate structures containing five-membered rings. A broad band centered at 3400 cm\(^{-1}\) is observed in the case of MCM-41 from the stretching vibrations of water adsorbed and silanol hydroxyl groups, along with another prominent band around 1630 cm\(^{-1}\) assigned to water deformation. The IR spectra of mesoporous molecular sieves modified with 3-aminopropyltriethoxysilane has bands around 3470 and 1634 cm\(^{-1}\) which are attributed to the stretching and bending frequencies, respectively, of the amino group in the SMCM-41. The spectra also reveal a band in the region 3000–2800 cm\(^{-1}\) characteristic of the presence of \(-(\text{CH})_3-\text{NH}_2\) group (supporting material). These observations indicate that the MCM-41 was indeed modified by 3-aminopropyltriethoxysilane. The degree of silylation achieved for SMCM-41 was determined by comparing the IR spectra in the region of the silanol groups before and after the treatment with 3-aminopropyltriethoxysilane.

Proflavine exists as unprotonated and protonated species depending upon the pH of the aqueous medium. Proflavine exists in the aqueous solution in three different forms according to the protolytic equilibria shown below.

![Equilibria Diagram]

In aqueous solution two pK values have been determined with pK\(_1\) = 0.2 and pK\(_2\) = 9.5 [28]. Commercially obtained proflavine hemisulfate is in the form of mono protonated proflavine which shows an absorption band at 445 nm and emission band at 510 nm in aqueous solution.

The properties of the guest molecules encapsulated in the channels depend upon the microenvironment of the pores of the mesoporous materials. The microenvironment remarkably influences the photophysical and photochemical processes of the encapsulated organic molecules. The diffuse reflectance UV–vis spectrum of proflavine in MCM-41 is shown in Fig. 2b; the absorption spectrum of proflavine monocation in aqueous solution is given as inset for comparison. Diffuse reflectance UV–vis spectrum of proflavine in MCM-41 shows maximum at 444 nm. No significant shift in the absorption maximum was observed as
compared to the spectrum in aqueous solution, even though the spectrum is a bit broader in the case of proflavine encapsulated in MCM-41. The broadness is presumably due to the presence of different sites in MCM-41. UV–vis DRS studies show that the proflavine molecules encapsulated are in monocationic form inside the channels of MCM-41.

Proflavine in silane modified SMCM-41 has an absorption band at 394 nm (Fig. 2a), which is 50 nm blue shifted as compared to that of proflavine in unmodified MCM-41. The absorption maxima for this spectrum is observed at 394 nm. The increase in the basicity is attributed to the presence of free amino groups inside the channels as shown in Scheme 1.

When proflavine under these conditions was encapsulated, the intracyclic ring nitrogen is deprotonated such that the dye exists in the neutral form, resulting in the observed blue shift. Laporta and De Silvestri reported that at highly basic pH, proflavine exists in the neutral form showing a maximum absorbance at 394 nm due to the deprotonation of the intracyclic nitrogen [35]. In fact, the ground state pK for this equilibrium is known to be 9.5 in aqueous solution. At highly basic solution above pH 11.0, the absorption and emission spectral properties of the dye show similar behaviour as reported [35]. A small shoulder in the absorption spectrum is also observed at 285 nm under highly basic conditions. The diffuse reflectance UV–vis spectra of proflavine in modified MCM-41 show a shift in the absorption maxima with a maximum at 394 nm and a shoulder at 285 nm which confirms that the proflavine molecules are present in neutral form inside the channels of SMCM-41 which are more basic and hydrophobic in nature.

### 3.1. Steady state fluorescence measurements

The fluorescence spectrum of PF encapsulated in MCM-41 is presented in Fig. 4a, along with that of PF in aqueous solution at pH 7.0 (inset). The PF/MCM-41 composite
exhibits characteristic emission band with maximum at 498 nm on excitation of the dye at 444 nm. The emission band is broadened as compared to the emission band in aqueous solution with a blue shift in the emission maxima from 510 nm to 498 nm. The near resemblance of the emission band of PF encapsulated in MCM-41 with that of PF in aqueous solution shows that PF exists as a monocation inside the channels of MCM-41. The channels and cavities in the mesoporous materials exhibit microheterogeneity in terms of polarity, acidity, electric field and restricted mobility. The novel photochemistry and photophysics observed for different guest molecules in these composite nanomaterials [16] are explained as a manifestation of these changes in these host environments.

The blue shift observed in the emission band in the case of PF/MCM-41 as compared to that in aqueous solution reflects that the local environment of the proflavine molecules in MCM-41 is less polar as compared to that in aqueous environment. The polarity of the MCM-41 host is comparable to that of the polarity of ethanol as inferred from the emission spectral data (Table 2). In ethanol, proflavine shows emission maximum at 496 nm whereas in MCM-41 the observed emission maximum is at 498 nm. A similar blue shift in the emission band is observed in the case of fluorescein encapsulated MCM-41 [36].

The fluorescence spectrum of PF in SMCM-41, presented in Fig. 4b is somewhat broad showing a maximum at 504 nm. The steady state absorption spectrum of PF in SMCM-41 is similar to that observed for neutral PF. Absorption and fluorescence spectra for neutral proflavine molecule in toluene show maxima at 397 nm and 479 nm, respectively [35]. The absorption spectrum of PF in toluene shows a maximum at 397 nm which is close to that of the neutral molecule in water (394 nm) and the blue-shifted fluorescence emission with a maximum at 479 nm is indeed observed. The absence of any blue shift in the fluorescence emission in silane modified MCM-41 suggests a very fast protonation of the intracyclic nitrogen in the excited state leading to the formation of the monocationic form. The observed emission is therefore attributed to the cationic form and not due to the neutral species as discussed later using lifetime measurements.

Excitation spectra of PF in MCM-41 and SMCM-41 are presented in Fig. 3a and b, respectively. The microenvironments in these two hosts are considerably different; in one case the proflavine molecules in MCM-41 host exist in a relatively acidic environment and in the SMCM-41 the guest molecule exists in a basic environment. The excitation spectra of PF obtained at very acidic pH (3.0) and also in a high basic pH (13.0) show maxima at 445 nm and 394 nm, respectively. The excitation spectrum of PF in MCM-41 shows maximum at 445 nm corresponding to the monocationic form. In the case of SMCM-41 the observed maximum is at 394 nm indicating that in the ground state PF exists in the monocationic form in MCM-41 and as neutral species in SMCM-41 which is further confirmed by the time resolved fluorescence measurements described below.
3.2. Time resolved fluorescence measurements

The fluorescence lifetime of proflavine measured in aqueous solution at pH 7.0 is found to be 4.92 ± 0.1 ns [35] and confirmed in the present study. The fluorescence decay curves of proflavine in MCM-41 do not fit single exponential decay and the decay curves are fitted satisfactorily with the biexponential function according to the following equation:

\[
I(t) = B_1 \exp(-t/\tau_1) + B_2 \exp(-t/\tau_2)
\]

where \(B_1\) and \(B_2\) are the pre-exponential factors and \(\tau_1\) and \(\tau_2\) are, respectively, the fluorescence lifetimes. The goodness of the fit is checked by the \(\chi^2\) values, which lie between 0.8 and 1.2, and the residuals are randomly distributed around zero. Proflavine in MCM-41 shows lifetime of 6.05 ns (85%) and 3.02 ns (15%). Scaiano et al. observed biexponential decay for 2,4,6-triphenyl pyrilium ion entrapped in MCM-41 [37]. The significant difference in the lifetime is attributed to the change of the local environment of proflavine in MCM-41. Encapsulation of proflavine in the pores of MCM-41 prolongs the lifetime of the excited state since the proflavine molecule experiences rigid inorganic walls which largely restrict the movement of the dye decreasing the rate constant for non-radiative transitions. Calzaferri et al. pointed out that the dyes in mesoporous silicates exist as organised systems in the channel structure sieves and the excitation energy of the molecules in the channels could be transported to the molecules on the surface, resulting in charge separation which prolonged the fluorescence lifetime [38]. The shorter lifetime component with a relative amplitude of 15% with a lifetime of 3.1 ns is attributed to the emission from the excited proflavine singlet state nearer the silanols (8–27% of silicon atoms is

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration of PF (w/w)</th>
<th>Absorption maxima (a) (nm)</th>
<th>Emission maxima (a) (nm)</th>
<th>Fluorescence lifetime (b) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF in water</td>
<td>(5.76 \times 10^{-6})</td>
<td>445</td>
<td>510</td>
<td>4.92</td>
</tr>
<tr>
<td>PF in ethanol</td>
<td>(5.76 \times 10^{-6})</td>
<td>456</td>
<td>496</td>
<td>4.61</td>
</tr>
<tr>
<td>PF-MCM-41</td>
<td>(2.71 \times 10^{-6})</td>
<td>444</td>
<td>498</td>
<td>3.02 (15%)</td>
</tr>
<tr>
<td></td>
<td>(1.06 \times 10^{-5})</td>
<td>444</td>
<td>498</td>
<td>3.11 (15.14%)</td>
</tr>
<tr>
<td></td>
<td>(6.31 \times 10^{-5})</td>
<td>444</td>
<td>498</td>
<td>2.95 (18.16%)</td>
</tr>
<tr>
<td>PF-SMCM-41</td>
<td>(1.08 \times 10^{-5})</td>
<td>394</td>
<td>504</td>
<td>0.72 (6.13%) (c)</td>
</tr>
</tbody>
</table>

PF – proflavine.

\(a\) Absorption and emission ±2 nm.

\(b\) Lifetime ±0.04 ns, amplitude ±5%.

\(c\) Fluorescence lifetime data at the emission maxima: 504 nm, average value of two independent measurements.
linked with pendant –OH groups \( \equiv \text{Si–OH in MCM-41} \), which quench the luminescence of the dye molecules. The hydroxyl group connected to the wall of MCM-41 and the high-energy vibration of these –OH groups effectively quench the luminescence of rare earth ions [39].

The fluorescence decay curves of proflavine in SMCM-41 do not fit single exponential decay. In SMCM-41 the decay of the emitting state of proflavine is triexponential with a lifetime of 0.73 ns (21.79%), 2.57 ns (43.24%) and 7.97 ns (34.97%) at 450 nm, 0.72 ns (6.13%), 2.34 ns (40.10%) and 6.42 ns (53.77%) at the emission maxima and 59 ps (−16.71%), 1.61 ns (44.92%) and 5.70 ns (71.79%) at 600 nm with the relative amplitudes given in the parenthesis. At longer wavelength, red edge region of the emission spectrum the fluorescence decay of PF in SMCM-41 exhibit an initial rise followed by a slow decay (Fig. 5) while in the case of the dye encapsulated in MCM-41 no such rise time is observed at any wavelength of the emission spectra. The observed rise time of the emission decay at the red edge of the emission spectrum corresponds to the excited state proton transfer reaction in the channel (vide infra). The growth observed in the fluorescence decay at the red edge of the fluorescence spectrum in SMCM-41 is suggested to be due to an excited state chemical process. The triexponential fit of the fluorescence decay curve reveals that the dye molecules are not

![Fig. 5. Fluorescence decay curves of PF in SMCM-41: (a) lamp profile, (b) decay monitored at 450 nm, (c) decay monitored at the emission maxima 503 nm, (d) decay monitored at 600 nm. Inset: (1) initial part of the fluorescence decay monitored at 450 nm, (2) initial part of the fluorescence decay monitored at 600 nm.](image-url)
homogeneously encapsulated in the channels of the silane modified MCM-41. Silylation reduces the number of OH groups in the channel thus transforming the original hydrophilic MCM-41 into a partially hydrophobic channel. In a more polar environment the lifetime is shorter as compared to that in non-polar medium. The longer lifetime observed in the case of PF in SMCM-41 as compared to that in MCM-41 is indicative of a more hydrophobic environment experienced by the guest molecules in the nanocavities of SMCM-41. The rise time corresponding to the protonation reaction shown in Scheme 2 is about 58 ps which is at the limit of the instrument used. A similar experiment carried out using a femtosecond upconversion technique showed the protonation reaction which is completed in about 60 ps. Encapsulation of proflavine on these two different mesoporous supports produces remarkable changes in the photophysical behaviour and is attributed to the environment the probe experiences; weak acid sites (in the case of MCM-41) and changes in the hydrophilicity/hydrophobicity of the surface in case of silane modified MCM-41. Silylation reduces the number of –OH groups, thus transforming the original hydrophilic MCM-41 into a hydrophobic modified MCM-41.

4. Conclusions

The molecular environment inside the mesoporous MCM-41 type composites was probed by incorporating proflavine in the nanochannels of the composites by impregnation method. The spectral properties of proflavine in MCM-41 are quite different when compared to that of silane modified MCM-41, SMCM-41. Proflavine molecule experiences the environment in MCM-41 which is similar to that in a solvent which retains the guest in monocationic form, whereas in modified MCM-41 the guest molecule in the basic and smaller pores exist in the neutral form. Our present study shows that the excited state properties of proflavine are sensitive to the nature of the mesoporous environment. In the present study we have also observed a novel excited state proton transfer reaction inside the nanocavities of mesoporous materials as probed by the picosecond time resolved fluorescence investigation.

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Appendix A. Supplementary data


References


