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Ciprofloxacin@SiO_2: Fluorescent nanobubbles[¶]

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Abstract. We report a new nanomaterial in which ciprofloxacin molecules are incorporated inside silica nanobubbles, denoted as ciprofloxacin@SiO_2. The material has been characterised using UV/Vis absorption spectroscopy, transmission electron microscopy, cyclic voltammetry, and emission spectroscopy. The material is stable and the freestanding particles can be precipitated and redispersed in several solvents. Confinement of the molecule is complete as leaching through the shell is minimal. The material behaves like free ciprofloxacin in solution; however, effects of confinement are manifested. Energy transfer reaction between ciprofloxacin@SiO_2 and Tb^{3+} was monitored by emission spectroscopy. The emission intensity decreased with metal ion exposure indicating selective electronic interaction.

Keywords. Ciprofloxacin; SiO_2 nanobubble; Tb^{3+}; fluorescence.

1. Introduction

Ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(piperazinyl)-quinolone-3-carboxylic acid] is a fluoroquinolone antibacterial agent, which is widely used in human and veterinary medicines.[1] It is one of the drugs used in the treatment of infections due to *Bacillus anthracis* (i.e., anthrax infections).[2] Because of this, study of fluoroquinolones is generating immense interest among scientists all over the world. There are studies on the modified pharmacological and toxicological properties of these drugs in the form of metallic complexes.[3–6] Structure of ciprofloxacin is shown in scheme 1 and consists of piperazine and pyridone moieties.

In this paper, we discuss the incorporation of ciprofloxacin inside a silica nanoshell. We undertook this research as part of our ongoing efforts on monolayer protected clusters[7] and core-shell nanoparticles.[8] There are a few studies in the literature on the incorporation of molecules, especially dye molecules, inside silica shells.[9–11] Advantage of such a system is that the molecules trapped inside the shell can be separated from unwanted interactions of the outside media. The main objective of most of these studies is the investigation of photochemical and spectroscopic aspects of the incorporated molecules.[9–12] Apart from these, there are a few other studies on the application of the incorporated molecules in fields such as sensing, optical switching, etc.[13–15] The

[¶]Dedicated to Professor C N R Rao on his 70th birthday

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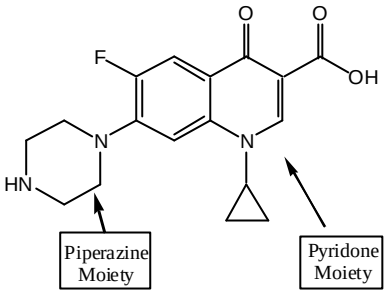

Scheme 1. Structure of ciprofloxacin molecule.

material obtained after incorporation of ciprofloxacin inside the silica nanobubble is designated as ciprofloxacin@SiO_2. We have characterised the material using UV/Vis absorption spectroscopy, cyclic voltammetry, transmission electron microscopy and emission spectroscopy. We have investigated the interaction of ciprofloxacin with a lanthanide ion. Lanthanide ions, especially terbium (III) and europium (III) show unique fluorescent properties when complexed with organic ligands.[16] Based on energy donation to lanthanides by the organic molecules, methods for their selective and sensitive determination have been developed.[17] Here our objective was to confirm that ciprofloxacin present inside the bubble behaves the same way as free ciprofloxacin. It was found that the emission intensity of the molecule decreased considerably by the addition of terbium (III), just as in free ciprofloxacin.

2. Experimental

Chloroauricacid ($HAuCl_4$) and trisodium citrate were purchased from CDH fine chemicals, India. (3-amino) propyl methyl diethoxysilane (APS), tetra methoxysilane (TMS) and Tb_4O_7 were purchased from Aldrich and were used without additional purification. Ciprofloxacin was prepared by our newly developed methodology.[18] It was also purchased from Fluka. Ethanol and 2-propanol were purchased from E Merck. Carbon tetrachloride was purchased from Ranbaxy Chemicals, India. Ultra pure water was used for all the experiments. UV-Vis absorption spectra were recorded using Perkin–Elmer Lambda 5 spectrometer. Emission spectra were measured using an F-4500 Hitachi spectroflourimeter. Particles were placed onto holey carbon films by dropping the suspension onto the grid and allowing it to dry; these were imaged using conventional bright-field transmission electron microscopy (Philips CM20, 200 KeV). Cyclic voltammetry data were obtained from an Electrochemical Analyser (CH Instruments Model 600A) in a standard three-electrode cell comprising of a Pt disk (area = 0·8 mm^2) as the working electrode, a platinum foil as the counter electrode and Ag/AgCl as the reference electrode.

2.1 *Synthesis of ciprofloxacin@SiO_2*

Gold nanoparticles of size 10–15 nm were prepared using the Turkevich[19] reduction method. In order to cover this gold particle with silica, a method adopted by Makarova *et*

al[10] was followed. To 200 ml of the gold sol, 1 ml of 1 mM propanolic solution of ciprofloxacin was added under vigorous stirring and was allowed to stand for 15 min so that complete complexation of ciprofloxacin on gold surface took place. Next, 1·5 ml of 1 mM freshly prepared solution of APS was added to it with vigorous stirring. This mixture of gold particles with APS was allowed to stand for around 15 min for complete complexation. A solution of active silica was prepared by adjusting the pH to 10–11 of a 0·54 wt% of sodium silicate solution by progressive addition of a cation exchange resin, Dualite C 225 – Na 14–52 mesh. 10 ml of active silica thus prepared was added to 200 ml of the surface modified gold sol. The resulting mixture was allowed to stand for one day, so that the active silica polymerises on the surface of the gold particle to form Au@ciprofloxacin@SiO_2.

Further growth of the silica shell was achieved by following the Stober method[20] and the particles obtained by this method were of 90 nm diameter. The solution thus obtained was centrifuged for around 1 h and the particles were collected which were repeatedly washed with 2-propanol to make sure that no ciprofloxacin was present on the surface of silica. This material was re-dispersed in about 100 ml of 2-propanol. To this solution, 25 ml of CCl_4 was added and stirred for around 48 h to remove the gold core.[21,22] The reaction between gold core and CCl_4 was monitored by UV-Vis spectroscopy. The dissolution of gold was confirmed by the disappearance of the gold plasmon peak and also from the colour of the solution (becomes colourless after the completion of the reaction). Formation of the bubbles was confirmed from the TEM image. The bubbles thus obtained were centrifuged for around six hours; the product collected was washed with propanol and water, and re-dispersed in water to yield ciprofloxacin@SiO_2. The material in the semi-dry form can be redispersed. It appears like a fluffy white powder. The solution is transparent to the naked eye.

3. Results and discussion

3.1 *Transmission electron microscopy*

TEM images of the ciprofloxacin@SiO_2 nanobubbles are shown in the figure 1. We can see particles of average shell diameter of around 15 nm (figure 1a), in agreement with the gold nanoparticles used in the synthesis. The shell morphology is largely spherical as expected. A close-up view of one particle is shown in figure 1b where a shell of < 2 nm thickness is visible. The particles are extremely beam-sensitive and they collapse upon prolonged exposure. Different studies of dye incorporation inside the silica shell by the same method have also reported nanobubbles of the same size.[9–11]

3.2 *Optical studies*

The absorption spectra of free ciprofloxacin, Au@ciprofloxacin@SiO_2 and ciprofloxacin@SiO_2 in the propanolic medium are shown in figure 2. Free ciprofloxacin shows three characteristic peaks around 225, 283 and 326 nm in alkaline media[23] (spectrum a). The absorption spectrum of Au@ciprofloxacin@SiO_2 shows (spectrum b) the characteristic plasmon absorption band of gold nanoparticles apart from the three ciprofloxacin peaks. As we can see there is a red shift in the plasmon from the typical value of 521 nm to 526 nm for the gold nanoparticles. We are attributing this to the adsorption of ciprofloxacin as well as the covering of silica on the nanoparticle surface; the latter being

more significant.[24] The spectrum c is that of ciprofloxacin@SiO_2 which shows only the peaks due to ciprofloxacin. A red shift seen in the case of ciprofloxacin@SiO_2 is very much expected since many ciprofloxacin molecules are present inside the confined volume of the nanoshell (as the surface of gold is covered by several molecules). A similar kind of red shift in the absroption spectrum is reported in the case of flourescein isothiocyanate incorporated inside the nanosphere.[9] Apart from UV/Vis we tried to characterise the material using nuclear magnetic resonance spectroscopy, but low concentration prevented us from getting a quality spectrum.

3.3 *Emission studies*

Emission characteristics of ciprofloxacin@SiO_2 were also studied. Free ciprofloxacin shows an emission peak around 448 nm[25] while ciprofloxacin@SiO_2 shows a red shifted

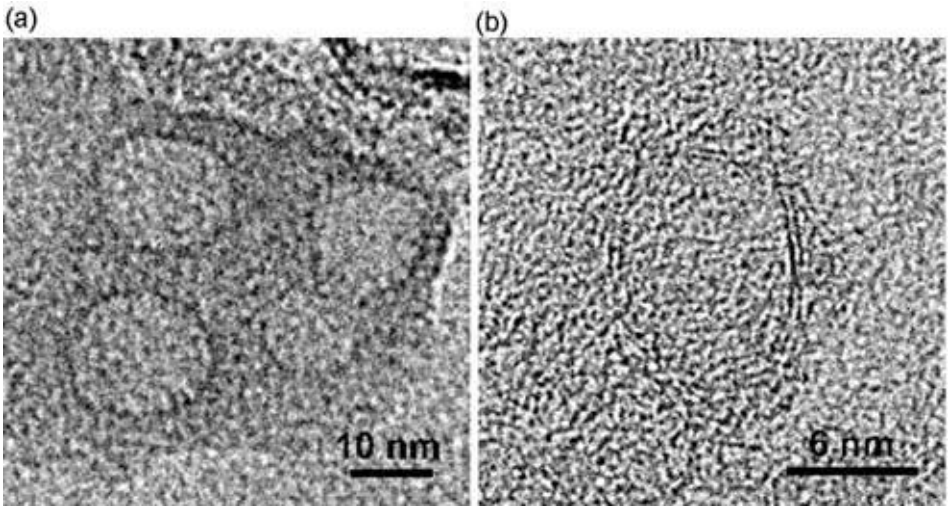

Figure 1. TEM images of ciprofloxacin@SiO_2. (**a**) is a large area image showing several particles, and (**b**) is a close-up of one particle.

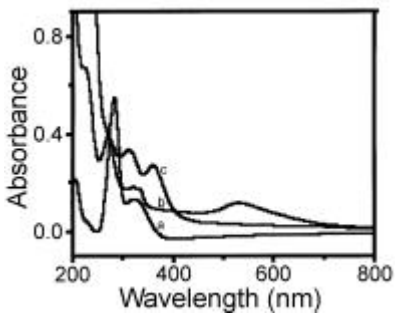

Figure 2. UV/Vis spectra of (a) free ciprofloxacin, (b) Au@ciprofloxacin@SiO_2, and (c) ciprofloxacin@SiO_2, in propanolic solution.

peak at around 458 nm (figure 3). This red shift in the emission wavelength may be due to changes in the environment of the drug molecule, such as changes in polarity. This kind of red shift was also found in the case of rhodamine 6G adsorbed on clay minerals and fluorescein isothiocyanate inside silica spheres.[7,26] In the case of ciprofloxacin@SiO_2, as the number of molecules in the vicinity increases, the excited state energy is lowered resulting in a red shift in the emission maximum. After the emission spectroscopic measurements, in order to confirm that ciprofloxacin is indeed inside the nano shell, we centrifuged the ciprofloxacin@SiO_2 solution, removed the bubbles and tested the centrifugate for ciprofloxacin. The solution did not show any fluorescence, confirming the fact that the molecule is indeed inside the silica shell. While preparing ciprofloxacin@SiO_2, before adding CCl_4, we washed Au@SiO_2 repeatedly with propanol so that there is no adsorbed ciprofloxacin on the silica surface.

When we mixed the ciprofloxacin@SiO_2 solution with Tb^{3+}, fluorescence intensity was quenched showing that charge transfer is taking place between ciprofloxacin and the terbium ion in the course of time. The quenching of fluorescence intensity of ciprofloxacin@SiO_2 shown in figure 4 was monitored after around 18 h. Previous reports

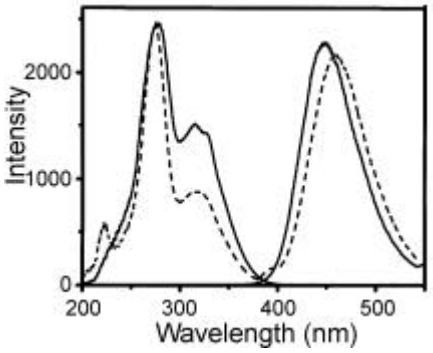

Figure 3. Excitation and emission spectra of free ciprofloxacin (solid line), and ciprofloxacin@SiO_2 (dotted line)

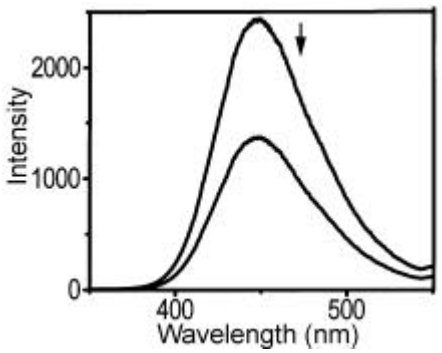

Figure 4. The quenching of fluorescence of ciprofloxacin@SiO_2 after the addition of Tb^{3+}. Top spectrum is the parent solution and bottom spectrum is the one after adding Tb^{3+}.

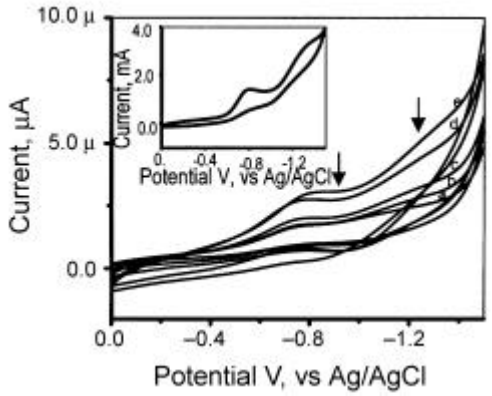

Figure 5. CV of ciprofloxacin@SiO_2, taken on a Pt electrode in DMSO/$TBAPF_6$ at different sweep rates (mV/s^{-1}). (a) 20, (b) 50, (c) 100, (d) 200, (e) 400. The reduction peaks are marked by arrows. The inset shows the CV of free ciprofloxacin under the same conditions.

of similar studies on the interaction between ciprofloxacin and terbium show a similar behaviour.[16] The silica shell which covers the ciprofloxacin is porous in nature, and it is expected that the Tb^{3+} ions go inside the shell.

3.4 *Electrochemical studies*

Electrochemical properties of ciprofloxacin@SiO_2 were studied by cyclic voltammetry. CVs of pure ciprofloxacin@SiO_2 taken on a Pt electrode in DMSO containing $TBAPF_6$ at five different sweep rates are shown in figure 5. Free ciprofloxacin shows two irreversible cathodic reduction peaks at –0·75 and –1·18 V (inset), which can be attributed to the reduction of piperazinyl and pyridone moieties[27] respectively. In the case of ciprofloxacin@SiO_2, however, the peak potentials are at –0·81 and –1·24 V respectively, with a shift of 60 mV towards the cathodic region. This may be due to the presence of the SiO_2 shell outside the ciprofloxacin molecule which retards the electrochemical reduction.

With increase in sweep rate, both the characteristic peak current and peak potential increase linearly which shows that the electrochemical reduction process is diffusion controlled in this solvent-supporting electrolyte system. The plot of peak current with square root of sweep rate is a straight line, passing through the origin, which also supports this (not shown in the figure).

The new material, ciprofloxacin@SiO_2, is stable for several months. In order to increase the solubility of the material, we modified it using stearic acid leading to ciprofloxacin@SiO_2@stearicacid. The material can be freely dispersed, precipitated quantitatively and stored for extended periods, without loss of fluorescence intensity.

4. Conclusions

In this paper we have reported a novel material, ciprofloxacin@SiO_2. It has been characterised using UV/Vis absorption spectroscopy, TEM, cyclic voltammetry and emission

spectroscopy. The energy transfer reaction between ciprofloxacin@SiO_2 and Tb^{3+} was monitored by emission spectroscopy. It was found that the fluorescence intensity decreased by the addition of Tb^{3+} indicating an energy transfer from ciprofloxacin to Tb^{3+}. Confinement effects are manifested in spectroscopy and voltammetry.

Acknowledgements

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