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## COMMUNICATION

## Solvent-dependent aggregation behavior of a new Ru(II)-polypyridyl based metallosurfactant<sup>†</sup>

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## Variation of the solvent polarity leads to the formation of vesicles and reverse vesicles of a newly synthesized amphiphilic Ru(II)-polypyridyl complex.

Amphiphilic molecules self-assemble to generate various interesting structures that differ in nature, size and shape. The nature of these self-assembled structures varies between miceller/reverse miceller structures, lamellae, ellipsoids, disks, cylinders and vesicles/reverse vesicles depending on the solvent environment, molecular architecture and concentration.<sup>1,2</sup> Among these various forms, vesicles composed of a spherical bilayer of amphiphiles containing an aqueous core have drawn much attention from a fundamental structural perspective, as well as for their potential applications in modeling cell membranes,<sup>3</sup> drug and gene delivery,<sup>4</sup> as nanoreactors,<sup>5</sup> templating nanomaterials,<sup>6</sup> gelation<sup>7</sup> and molecular recognition.<sup>2a</sup> Reverse vesicles are composed of a reverse bilayer shell that encapsulates solvent of low polarity, where hydrophobic portions of the precursor amphiphiles are exposed to the low polar solvent both in the core and in the exterior.8 These are of great fundamental interest because they have many impending applications like normal vesicles, e.g., encapsulation and controlled delivery of hydrophobic solutes.9 To fully realize the promising aspects mentioned above, a detailed understanding of the structural features of normal vesicles and reverse vesicles is necessary. However, studies in which metalloaggregates have been used for vesicle formation are scarce in the literature and more importantly these aggregates have been studied mainly in aqueous solution.<sup>10</sup> Thus, studies with reverse vesicle formation in non-polar organic solvents are even scarcer.<sup>11</sup> Complexes containing the  $Ru(bpy)_3^{2+}$  (where bpy = 2,2'-bipyridine) unit as the building block are of wide interest due to their distinct coordination geometry, stability in the ground and in the photoexcited states, and rich redox/photophysical properties.<sup>12</sup> Though, reports on the aggregation behavior of amphiphilic complexes containing  $Ru(bpy)_3^{2+}$  as the polar head group and an aliphatic long chain as the lipophilic tail are there, examples

on the use of such metallosurfactants for the formation of vesicles or reverse vesicles are uncommon.<sup>13</sup> Such structures are of immense importance for their application potential as photodynamic therapeutic reagents. Elsevier and Eiser have two earlier reports, which reveal the formation of inverted micelles/vesicles using Ru(bpy)32+ derivatives;13 however, to the best of our knowledge there is no report on normal vesicles. In this communication, we are reporting a new metallosurfactant (1) using the Ru(II)-polypyridyl core as the hydrophilic head group with each bpy-ligand functionalized with a hydrophobic C<sub>18</sub> hydrocarbon tail (Scheme 1). This complex is found to form normal vesicles in water and reverse vesicles in non-polar solvents like toluene and cvclohexanean observation that is unique in the literature. These vesicles were characterised by dynamic light scattering (DLS), static light scattering (SLS), atomic force microscopy (AFM) and transmission electron microscopy (TEM) studies.

Complex 1 (Ru(L<sub>3</sub>)<sub>3</sub>Cl<sub>2</sub>) was synthesized by reacting L<sub>3</sub> and RuCl<sub>3</sub>·3H<sub>2</sub>O in appropriate mole ratio in a mixed solvent (ethanol–dioxan, 1:1, v/v) medium at 90 °C and the desired purity was achieved by column chromatography.† Complex 1 was expected to exhibit aggregation behavior in polar as well as in nonpolar solvents due to its amphiphilic nature. It was found to form aggregates on dropwise addition of water to the methanol solution of 1 and the final solvent combination was adjusted to CH<sub>3</sub>OH:H<sub>2</sub>O = 1:9 (v/v) (~10<sup>-6</sup> M). DLS studies with this showed aggregates having distribution of varying hydrodynamic diameters, with a mean diameter (*D*<sub>h</sub>) of 230 nm (Polydispersity index (PDI) = 0.18) (Fig. 1A). The larger hydrodynamic diameter revealed that these aggregates



Scheme 1 Molecular structure of compound 1 and cartoon representation of aggregates in different solvents.

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**Fig. 1** Size distribution of **1** in (A) methanol–water (1:9; v/v), (B) toluene and (C) cyclohexane medium. Insets: Guinier plot obtained from SLS studies in respective solvent medium.

were vesicles not micelles, as for micelles the diameter should be in the order of  $3-7 \text{ nm.}^{14}$ 

Static light scattering (SLS) measurements were also performed in methanol–water medium (1:9, v/v) with varying angles from 30°–150° and radius of gyration ( $R_g$ ) was evaluated from the slope of the Guinier plot (inset, Fig. 1A).<sup>15</sup> † The average  $R_g$  value obtained for these aggregates is 116.6 nm and the  $R_g/R_h$  ratio (where  $R_h = 1/2 D_h$ ) is 1.01, which is very close to the value for a spherical vesicle.<sup>16</sup>

Vesicle formation was also confirmed by AFM studies. Fig. 2A shows the morphology of the self-assembled vesicles and this enabled us to calculate the diameter of the collapsed dry aggregates. AFM images further revealed that the aggregates were ellipsoid instead of spherical. The deposited droplet over the mica surface did not undergo instant drying; instead there was a moving drying front which allowed these vesicles to orient along its direction and accounts for the ellipsoid shape. AFM cross-sectional analysis also revealed that the length, width, aspect ratio and diameter to height ratios are in the order of 174-465 nm, 108-347 nm, 1.25-1.6 and 3.9-5.7 respectively.<sup>†</sup> The diameters of these vesicles are thus larger than the heights of the vesicles, which indicates flattening upon being transferred from solution to the mica surface.<sup>†</sup> Removal of solvent molecules from the hollow spheres during the drying process and the high local force applied by the AFM tip could have also contributed to this. Thus these structures are robust, yet soft enough to deform upon drying.

The spherical nature of these vesicles was further confirmed by TEM studies. A small drop of the methanol–water (1:9; v/v) solution was deposited on a carbon coated copper grid and air dried. Fig. 3A shows the 2D projection of the polydispersed spherical vesicles. These vesicles were in the 100–400 nm diameter range and belong to medium size vesicles.<sup>2a</sup> These images, on staining with 1% uranyl nitrate solution, showed a darker cell wall with a thickness of 7–8 nm (Fig. 3A). The length of the fully extended individual molecule of **1** is ~3.5 nm and this suggests that the vesicle wall is a bilayer and these vesicles are unilamellar and hollow in nature. Again fusion of two nearby spheres and fission of the large spheres to



Fig. 2 AFM images of cast films deposited on mica of 1 in (A) methanol: water (1:9; v/v), (B) toluene and in (C) cyclohexane.



**Fig. 3** Unstained TEM images of **1** in (A) methanol:water (1:9; v/v), (B) toluene and in (C) cyclohexane. Inset (A):  $UO_2^{2+}$  stained TEM image in methanol:water (1:9; v/v) showing cell wall thickness. Inset (C): magnified unstained TEM image showing cell wall thickness.

form smaller spheres as observed from the TEM images also support the vesicular nature of these spherical structures.†

Complex 1 with the  $Ru(bpy)_3^{2^+}$ -core as the polar head group and three symmetrical hydrophobic tail groups is expected to adopt a cone shape geometry. <sup>13b</sup> Such cone shaped complexes are expected to form inverted micelles or vesicles in nonpolar medium. We checked such a possibility in toluene and cyclohexane. The solubility of 1 in toluene was found to be higher than in cyclohexane, which could be ascribed to the higher polarity of toluene. In the midst of the apolar environment the apolar aliphatic chains are expected to be exposed to the solvent medium with the hydrophilic headgroup situated at the core of the bilayers that face each other (Scheme 1). This is further supported by the fact that  $Ru(bpy)_3^{2^+}$  derivatives are not soluble in apolar solvents like toluene and cyclohexane.

The DLS study of a freshly prepared solution of 1 ( $\sim 10^{-6}$  M) in toluene and cyclohexane revealed that mean  $D_{\rm h}$  for these molecular aggregates are 308 nm (PDI = 0.35) and 229 nm (PDI = 0.19), respectively (Fig. 1B and C).  $R_{\rm g}$  values obtained from SLS experiments for these aggregates are 166.3 and 121.4 nm (inset, Fig. 1B and C). Thus the  $R_g/R_h$  values are 1.08 and 1.06 in toluene and cyclohexane, respectively. These values are very close to unity and corroborate the formation of reverse vesicles.<sup>17</sup> Smaller size of the reverse vesicles in cyclohexane could be accounted if one considers that in media of lower polarity, the polar head set to tighter inverted vesicles. While toluene, being more polar among these, is expected to penetrate the vesicular membrane better and cause enhancement of the vesicle size. The mean hydrodynamic diameters of complex 1 in toluene and in toluene-methanol mixture of varying proportion (e.g. 25:1 and 25:4 (v/v)) were found to be 308, 61 and 39 nm respectively.<sup>†</sup> This further confirms the proposition that with the increase in polarity of the medium the interaction of the amphiphiles becomes more flexible and thus the rigidity of the inverted vesicles decreases, and finally they become soluble in the mixture of the solvents.

Results of the AFM experiments with cast films on mica confirm that complex **1** forms aggregates in toluene and cyclohexane solutions. The size of the reverse vesicles obtained from the toluene solution was found to vary between 270 and 530 nm in length and 170 and 320 nm in width, while the aspect ratio varied from 1.35-1.56 (Fig. 2).† Cross-section analysis of the typical structures revealed large ratios of diameter to height (4.4–6.8). In the case of cyclohexane solution, length, width, aspect ratio and diameter to height ratios are in the order of 134-333 nm, 117-280 nm, 1.2-1.5 and 3.7-6.7, respectively.† Thus AFM results confirmed that



Fig. 4 Emission spectra of absorption matched rhodamine B (A) in methanol-water (1:9, v/v) solution and in the presence of vesicles; (B) in water and in the presence of reverse vesicles in toluene solution of complex 1.

the reverse vesicles formed in both toluene and cyclohexane had flattened shape with rounded edges and the hollow feature of the original inverted vesicle. The reverse vesicles appeared with an ellipsoidal instead of a spherical shape, as during the drying process of the deposited droplet, solvents were not dried instantly and there was a moving drying front that allowed these reverse vesicles to orient along its direction and accounts for the ellipsoid shape. These reverse vesicles were found to be stable even in the dry state and sizes thus obtained from AFM studies matched well with those of the DLS study.

More convincing information about the reverse vesicles was obtained from TEM images. The mean diameters of the aggregates obtained from TEM were 250 and 209 nm, respectively, for toluene and cyclohexane solutions (Fig. 3B and C). These values are lower than that of  $D_h$  values obtained from the DLS method. This is understandable if we consider that DLS determines the hydrodynamic diameter, while TEM determines the radius of the collapsed dry aggregates. The wall thickness of the reverse vesicle was found to be ~ 7 nm for both instances (Fig. 3B and C), which indicated that the reverse vesicles formed by complex 1 either in toluene or in cyclohexane were of bilayer thickness and these structures were unilamellar.

In order to check the dye encapsulation property of these vesicles and reverse vesicles, these aggregates were generated in the presence of rhodamine B (  $\sim 10^6$  M). The emission property of rhodamine B in a vesicle/reverse vesicle solution was compared with that of a pure rhodamine B solution having comparable absorbance value at  $\lambda$  551 nm. The emission intensity recorded for rhodamine B with vesicles in methanolwater medium and with reverse vesicle in toluene/cyclohexane solution was found to be appreciably quenched as compared to that of free rhodamine B in water solution (Fig. 4 and ESI<sup>†</sup>). This luminescence quenching is typically the result of the encapsulation of the dye molecules inside these aggregates, which lead to an increase in local concentration and thus the self-quenching process (Fig. 4 and ESI<sup>+</sup>).<sup>18</sup> In reverse vesicles the effect of quenching of the dye was much higher compared to the vesicles. Presumably, in reverse vesicles the polar dye molecules are trapped in the reverse bilayer and more organised upon interaction with the polar head groups, whereas in vesicles the dye molecules are in the interior and less organized.

In summary, the novel  $Ru(bpy)_3^{2+}$  based symmetrical amphiphilic complex 1, containing three  $-C_{18}H_{37}$  chains at equilateral positions, was synthesized and characterised by

different analytical techniques.† Complex 1 formed vesicles in polar aqueous-methanol (9:1; v/v) medium and reverse vesicles in nonpolar medium like toluene and cyclohexane. These vesicles and reverse vesicles were characterised by DLS, SLS, AFM and TEM studies. AFM and TEM confirmed the spherical and hollow nature of these aggregates. Aggregate formation was also confirmed by dye encapsulation studies in respective solutions.

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