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Robust molecular micro-capsules for encapsulating and releasing hydrophilic contents†

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The hydrophobic–amphiphilic self-assembly approach has been employed to prepare molecular micro-capsules simply by cooling down an emulsion, in a hot polar solvent medium, of a melted compound having two very well-distinguished units, both with highly non-polar and hydrophobic characteristics. The resulting micro-capsules are very stable and robust both in suspension and under dry conditions. Further, such micro-capsules can effectively encapsulate hydrophilic compounds which can later on be easily released upon the application of UV-light.

A key focus of attention in nanoscience is currently based on constructing large-scale systems of nano-structured materials that give rise to assemblies of high technological interest.[1] In such materials, the properties are mainly driven by the hierarchically organized assemblies, rather than the individual components. One particular case of micro-assemblies which raise an increasing significance is hollow spheres, also called capsules. They allow for the encapsulation of materials to protect them from the environmental influences or for the confinement of chemical reactions. An unlimited number of examples of encapsulation can be found in Nature, and the appeal of micro-capsules is expanded to a wide range of applications in the pharmaceutical, cosmetic, food, textile, adhesive and agrochemical industries. Especially interesting are stimuli-responsive capsules (*i.e.*, smart capsules) that are able to release the encapsulated substances triggered by external stimuli such as a pH change, light or temperature.[2]

It is well-known that organic-based capsules can be prepared by self-assembly of (phospho)lipids that aggregate in aqueous solutions owing to their amphiphilic nature forming bi-layered structures, so-called vesicles. These capsules can be further stabilized with polymerizable groups that give rise to interconnections between the molecules *via* covalent bonds and thus stabilize the shell-forming membrane.[3] However, in the most recent years, the use of macromolecules to prepare robust capsules has been more extensively explored employing a variety of techniques such as amphiphilicity strategy, polyelectrolyte layer-by-layer, phase separation or polymerization.[4,5]

Very recently, the so-called "hydrophobic–amphiphilic" approach was developed to prepare molecular micro-scale objects with unprecedented shapes (*i.e.*, flowers, cones, fibers, *etc.*).[6] This method was based on precipitating in a mixture of solvents a molecule bearing two very well-distinguished units, but both highly non-polar and hydrophobic, consisting of a fullerene derivatized with long alkyl chains. The van der Waals forces between the aliphatic chains and $\pi\cdots\pi$ interactions between the fullerene units determined the final assemblies. Later on, the method was applied to a different system: a perchlorotriphenylmethyl radical (PTM) moiety functionalised with three long alkyl chains.[7] In this case, the intermolecular $Cl\cdots Cl$ interactions of the PTM heads together with the $CH_2\cdots CH_2$ interactions of the alkyl chains drove the formation of compact functional assemblies with unusual shapes.[8]

In this communication, we synthesize a new polychlorotriphenylmethane (αH-PTM) derivative, compound **1**, bearing only two long alkyl chains at the *para* and *meta* positions of the phenylvinylene ring. This results in the lowering of the melting point of the compound down to 48 °C, which is abnormal for a polychlorinated aromatic derivative, and the possibility of interdigitation of the two alkyl chains in its supramolecular assemblies. We show that this hydrophobic molecule forms an emulsion in hot polar solutions that leads upon cooling to formation of micro-capsules without the need for carrying out any additional step. Further, it is demonstrated that such assemblies are very stable and robust and can be exploited to encapsulate polar molecules, which can then be neatly released upon UV irradiation.

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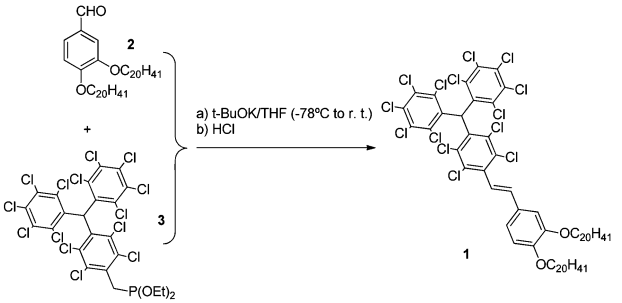

Scheme 1 Synthesis of **1**.

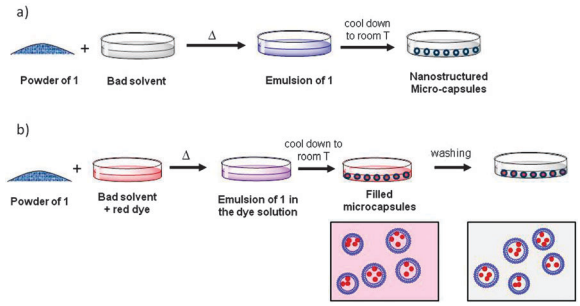

Fig. 1 Schematic procedure followed to form capsules of **1** (a) and to encapsulate the dye in them (b).

Compound **1** was synthesized in a 61% yield by a Wittig–Horner–Emmons reaction between the corresponding benzaldehyde **2** and the αH-PTM phosphonate **3**[9] (Scheme 1).

The optimised procedure followed to prepare micro-capsules of **1** consisted of preparing a suspension (1.4 mM) in THF–water (2.5/1) and heating it at 65 °C. This resulted in the melting of the suspended compound (mp. 45 °C) and thus the formation of an emulsion of melted **1** in such a polar solvent medium. After 5 minutes, the mixture was rapidly cooled down to room temperature leading to the final supramolecular assemblies. This procedure is schematically depicted in Fig. 1a.

To visualize the resulting supramolecular organizations, field emission scanning electron microscopy (FE-SEM) as well as transmission electron microscopy (TEM) images were acquired. Fig. 2 (top) shows the SEM images of the assemblies of **1** after being deposited on a silicon substrate, where spherical and regularly distributed hollow capsules can be clearly observed. Additionally, the TEM images of the particles deposited on a lacey carbon grid (Fig. 2, bottom) further confirmed their hollow nature. The cortex of capsules has an approximate thickness of 60 nm, which corresponds to around 15 molecular layers as analyzed by the X-ray characterization (see below). The size and distribution of the capsules were estimated by light scattering (LS) which gave an average diameter of 0.7 μm (Fig. S1, ESI†). It is important to highlight here that, in contrast to conventional vesicles that are stable in the solution where they are produced but collapse immediately on solid supports after solvent evaporation, the capsules of **1** once formed are stable and robust even when the mother solution is dried.

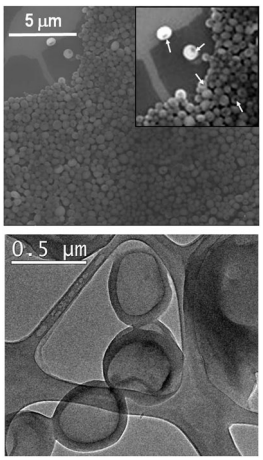

Fig. 2 (top) The SEM image of the capsules of **1** deposited on a silicon substrate. (inset) Zoom of the image showing with white arrows hollow capsules damaged by e-beam. (bottom) The TEM image of the micro-capsules of **1** on a lacey carbon grid.

In order to gain a better understanding of the supramolecular structure of the micro-scale objects formed by **1** small-angle X-ray scattering (SAXS) characterization was performed. The SAXS spectra of the assemblies of **1** at room temperature exhibit diffraction patterns that are attributed to the reflections (001) to (00*n*) with a *d* spacing value of *ca.* 5.1 nm that indicates a lamellar layered structure (Fig. S2, ESI†). Taking into account the approximate length measured for the extended molecules (~42 Å) and the diameter of αH-PTM moieties (~10 Å), for every layer a complete interdigitation of the alkoxy chains and a face-to-face interaction between the αH-PTM moieties are expected (Fig. S3, ESI†). Therefore, this confirms that the Cl···Cl and van der Waals interactions between the aliphatic chains are the driving forces for the supramolecular ordering of the micro-capsules formed in such a polar solvent medium.

To demonstrate the capability of the capsules of harboring hydrophilic guests, the red dye Rhodamine B or AlexaFluor-568® was dissolved in the THF–water mixture (2.5/1) (c = 0.25 mM) in the micro-capsule formation process (Fig. 1b). The fabrication process was carried out as before, although an additional washing step was introduced at the end in order to remove the non-encapsulated dye.

By optical fluorescence microscopy it is possible to observe the capsules and the dye taking advantage of the intrinsic fluorescence of **1** that shows a strong emission band at 465 nm when irradiated at 305 nm (Fig. S4, ESI†), and that of the red dye that emits at 550 nm (in the case of Rhodamine B, λ_{exc} = 510 nm) or 603 nm (in the case of AlexaFluor-568®, λ_{exc} = 510 nm). Fig. 3 shows the fluorescence microscope images of the capsules filled with Rhodamine B once deposited on a quartz slide. The micro-capsules are visible when using the transmitted white light. Once irradiated with green light (546 nm), the red fluorescence of the dye can be visualized at the same position where the capsules were seen. Furthermore, the micro-capsules switched to blue when the UV-light (305 nm) was employed for 2 seconds due to the emission of **1**. These results unambiguously confirmed the presence of the dye which was located in the same place as the micro-capsules.

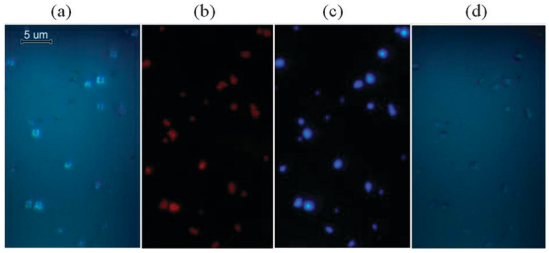

Fig. 3 Microscope images of micro-capsules of **1** deposited on a quartz slide with: (a) transmitted white light, (b) irradiation with green light (mercury lamp, 546 nm), (c) irradiation with UV light (mercury lamp, 305 nm) for 2 seconds and (d) transmitted white light upon irradiation for 10 s at 305 nm.

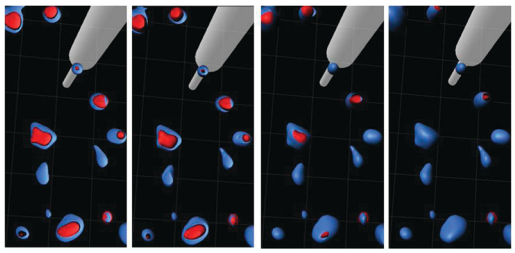

Fig. 4 Superposition of image captures of some cross-sections of micro-capsules of **1** (blue) with AlexaFluor-568® in their lumen acquired with two channels: the red channel (λ_{exc} = 561 nm; λ_{em} = 585–670 nm) where the dye emits; the blue channel (λ_{exc} = 476 nm; λ_{em} = 500–550 nm) where the micro-capsules of **1** emit.

However, after exposure for 10 seconds to UV light, the capsules are shattered due to the disassembling of the molecular units originating from a photochemical cyclization of **1** leading to a phenanthrene derivative;[10] as indicated by MALDI-ToF analysis and UV-Vis measurements (Fig. S5, ESI†).[11] As seen in the image taken after a longer UV-irradiation (Fig. 3d) the capsules are not structured anymore although some material is observed at the same positions where the original capsules were located.

To demonstrate that the dye was encapsulated within the lumen of the micro-capsules and was not deposited around them, confocal microscopy experiments were carried out. These experiments were performed using AlexaFluor-568® due to its enhanced photostability. Fig. 4 shows the confocal microscope images acquired at different planes separated by 0.04 μm using two – red and blue – channels. In this figure, images acquired using the red channel (λ_{exc} = 561 nm; λ_{em} = 575–670 nm) and the blue channel (λ_{exc} = 476 nm; λ_{em} = 500–550 nm) are found to overlap. Note that irradiating the sample at 476 nm did not damage the assemblies since compound **1** does not absorb at this wavelength. These images clearly demonstrate that capsules have been successfully filled within their lumen with a solution of the polar dye.

Experiments to release the micro-capsules by UV irradiation were also performed. The absorption and fluorescence spectra of the solvent from a suspension of micro-capsules of **1** filled with AlexaFluor-568® were registered before and after 10 seconds of UV irradiation at 305 nm. It was found that only after light irradiation the optical characteristics of the dye were detectable in the solvent media, confirming that it had been liberated (Fig. S6 and S7, ESI†).

In summary, it has been shown that the supramolecular hydrophobic–amphiphilic approach can be applied to fabricate robust molecular micro-capsules following a very simple process based on forming an emulsion of the melted compound **1** and solidifying it in a polar solvent medium by lowering the temperature. Such micro-capsules can effectively encapsulate hydrophilic compounds which can later on be easily released upon the application of UV-light that shatters the micro-capsules due to the disassembling of molecules. The fabrication of stable smart capsules based on molecules with the hydrophobic–amphiphilic approach offers an elegant alternative to the most common strategies currently used for preparing organic capsules which employs polymers or polymerizable monomers as building blocks, and thus brings novel perspectives to this field.

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11 A peak corresponding to $[M-HCl]^+$ appeared in the MALDI-ToF spectra and a new absorption band at around 300 nm, typical of phenanthrenes, was observed in the UV-Vis spectra.