Core-shell nano-architectures: the incorporation mechanism of hydrophobic nanoparticles into the aqueous core of a microemulsion

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Abstract
This work presents an in-depth investigation of the molecular interactions in the incorporation mechanism of colloidal hydrophobic-capped nanoparticles into the hydrophilic core of reverse microemulsions. $^1$H nuclear magnetic resonance (NMR) was employed to obtain molecular level details of the interaction between the nanoparticles capping amphiphiles and the microemulsion surfactants. The model system of choice involved oleic acid (OAC) and oleylamine (OAM) as capping molecules, while igepal-CO520 was the surfactant. The former were studied both in their ‘free’ state and ‘ligated’ one, i.e. bound to nanoparticles. The latter was investigated either in cyclohexane (micellar solution) or, in water/cyclohexane microemulsions. The approach was extremely useful to gain a deeper understanding of the equilibria involved in this complex system (oleic acid capped-Bi$_2$S$_3$ in igepal/water/cyclohexane microemulsions). In difference to previously proposed mechanisms, the experimental data showed that the high affinity of the capping ligands for the reverse micelle interior was the driving force for the incorporation of the nanoparticles. A simple ligand exchange mechanism could be ruled out. The collected information about the nanoparticle incorporation mechanism is extremely useful to develop new synthetic routes with an improved / tuned coating efficiency, in order to tailor the core-shell structure preparation.

KEYWORDS:
Reverse micelles; nanoparticles synthesis; ligand-exchange; paramagnetic relaxation enhancement; intermolecular interactions; liquid-state NMR

ABBREVIATIONS:
CHX: cyclohexane; CMC: critical micelle concentration; DQF-COSY: double quantum filtered correlation spectroscopy; NMR: Nuclear Magnetic Resonance; NOESY: nuclear overhauser effect spectroscopy; OAC: oleic acid; OAM: oleylamine; SI: Supporting Information; TEM: transmission electron microscopy; TEOS: tetraethyl orthosilicate; TMS: tetramethyl silane; TOCSY: total correlation spectroscopy.
1. Introduction

Core-shell nano-architectures have attracted great interest for their high added value applications [1–9], since the formation of shells around colloidal nanoparticles yields composites with significantly different properties, when compared to those of their core (e.g. increased stability, adhesion, surface area, magnetic and optical properties). In the literature, several approaches are reported for shell fabrication, such as surface chemical reactions, precipitation of inorganic salts, reverse micro-emulsion formation, sol-gel and seed-mediated growth processes [3]. All these synthetic strategies have both advantages and drawbacks, each of them resulting in a different shell uniformity, thickness and composition. Procedures that employ preformed nanoparticles and make use of reverse micelles as nano-reactors for shell growth, are widely considered to be among the most efficient approaches to obtain a highly homogeneous coating, especially with silica [10–19].

In particular, the igepal/cyclohexane/water microemulsion is considered to be the most efficient technique. Both hydrophilic [18] and hydrophobic [13–15,17] nanoparticles can be coated, resulting in monodisperse core-shell nanostructures with tunable shell thickness. Recently, some of the authors have reported the synthesis of core/shell CoFe$_2$O$_4$/SiO$_2$ nanoparticles, based on the igepal/cyclohexane/water microemulsion, starting from both hydrophilic [20] and hydrophobic [21] nanoparticles. However, while on the one hand a hydrophilic nanoparticle naturally tends to enter the aqueous core of the reverse microemulsion, on the other a hydrophobic nanoparticle should be expected to have the tendency to disperse in the nonpolar solvent. Thus, the coating process, which takes place inside the microemulsion core, should be inhibited for the latter. Surprisingly, the coating process was found to be more efficient in the case of hydrophobic nanoparticles. Such an unexpected behavior aroused much interest in the scientific community, but only few authors proposed a theory: Selvan at al. [16] suggested the exchange of hydrophobic capping agents by igepal, Darbandi et al. [22] by tetraethyl orthosilicate (TEOS), while Zhang and coworkers [14] proposed a mechanism involving the tail-to-tail intercalation of oleic acid (OAC) in the nanoparticles’ capping layer with igepal CO520, and the subsequent interaction of the igepal’s polar head groups with water to form an external water shell. In any case, direct evidence for the proposed mechanisms was not provided. Recently, Koole et al. [13] reported convincing experimental data from an in-depth time-resolved fluorescence spectroscopy investigation. They showed that both igepal and hydrolyzed TEOS do replace the original hydrophobic amine ligands of quantum dots, facilitating the successive transfer of the latter into the hydrophilic interior of the reverse microemulsion. They have also shown that, the stronger the ligand nanoparticle binding, the more hindered is the replacement by TEOS or igepal. However, this model
only describes the mechanism from a phenomenological point of view, but no details are given about the molecular interactions involved.

The present work aims at elucidating the mechanism for hydrophobic nanoparticles incorporation into the hydrophilic core of reverse microemulsions, with the inter-molecular interactions involved between the capping ligands and the non-ionic surfactant igeval CO-520 being our focus. Liquid-state Nuclear Magnetic Resonance spectroscopy (NMR) has been the method of choice. First, a careful analysis was carried out on the interactions between free ligands (i.e. in the absence of nanoparticles) and either the igeval reverse micelles (in cyclohexane) or the corresponding liquor ammonia and water microemulsions. The collected information was fundamental when, following the same approach, OAC-capped Bi$_2$S$_3$ nanoparticles were used in the successive stage. Besides monitoring chemical shift and multiplicity variations for selected $^1$H-NMR resonances, the MnCl$_2$ was also employed as a paramagnetic probe to estimate the insertion depth of the capping agents in the igeval’s micelles or in the microemulsions. The diamagnetic Bi$_2$S$_3$ has been the model-system of choice instead of the previously employed CoFe$_2$O$_4$, due to the ferrimagnetic properties of the latter, that results in an extreme NMR lines broadening, making the system difficult to investigate. The chosen nanoparticles are shown to be silica-coated to the same degree as the cobalt ferrite, thus representing a valuable model to get more general information about the aforementioned incorporation mechanism. The NMR approach was found to be capable of providing fundamental details about the complex equilibria involved in this kind of synthesis, being not only interesting for the specific system here investigated, but also as a more general route to compare different ligands and/or surfactants before undertaking the synthesis trials.

2. Materials and methods

2.1. Materials

Cyclohexane (CHX) was purchased from Panreac (Barcelona, Spain) at PA grade, while all the other chemicals were purchased from Sigma-Aldrich (St.Louis (MO), USA). In particular, polyoxyethylene (5) nonyl phenol ether, igeval-CO520 with an average $M_n$ of 441 g/mol (hereinafter referred to simply as igeval), oleic acid (OAC; 90%), oleylamine (OAM; 70%), tetraethyl orthosilicate (TEOS; 98%), ammonia (NH$_3$ 30% w/w), MnCl$_2$ (98%) and cyclohexane-d$_{12}$ (99.6 atm.% D) were employed.

2.2. Hydrophobic inorganic core: CoFe$_2$O$_4$ and Bi$_2$S$_3$ nanoparticles synthesis
OAC-OAM capped CoFe$_2$O$_4$ and OAC-capped Bi$_2$S$_3$ nanoparticles were prepared by following the procedures described in detail elsewhere [21,26,27].

2.3. Hydrophilic silica shell: core/shell CoFe$_2$O$_4$/SiO$_2$ and Bi$_2$S$_3$/SiO$_2$ nanoparticles synthesis

Silica coating of the hydrophobic nanoparticles was carried out as described in detail elsewhere [14,20,21]. Specifically, 0.56 mmol of the non-ionic surfactant igepal were dispersed in 4 mL of CHX by sonication. Then, 300 µL of a CHX dispersion of hydrophobic nanoparticles (0.8 mg/mL for CoFe$_2$O$_4$ and 8 mg/mL for Bi$_2$S$_3$), previously sonicated for a few minutes, were added, and the mixture was vigorously stirred at room temperature for 15 min. TEOS (20 µL) was added, and the resulting mixture stirred for 30 min, prior to liquor ammonia (35 µL) addition. The reaction was allowed to proceed at room temperature for 24 h under stirring.

2.4. Transmission electron microscopy

The colloidal suspensions were dropped on carbon-coated copper grids and observed in Bright Field mode with a TEM (JEOL 200CX), operating at 200 kV. Particle size distribution was calculated on about 200 nanoparticles. Different images, obtained in bright field mode, were analyzed with Adobe Photoshop determining the diameters manually.

2.5. Nuclear magnetic resonance spectroscopy

Five different groups of NMR samples were prepared: (i) cyclohexane solutions, (ii) liquor ammonia microemulsions, (iii) water microemulsions, (iv) paramagnetic probe microemulsions, and (v) OAC-capped Bi$_2$S$_3$ nanoparticles dispersions. Cyclohexane (90% non-deuterated + 10% perdeuterated for frequency-lock) was always employed as solvent and all the solutions have been prepared at room temperature (~298K).

(i) First, starting from a concentrated stock solution of either igepal, or OAC, or OAM, differently diluted samples were prepared in the 0.2-140 mM range for critical micelle concentration (CMC) determination. Then, OAC (2 mM) and OAM (2 mM) were investigated separately, or as a 1:1 mixture. In particular, they have been studied either in the absence or in the presence of igepal at two different concentrations, namely, 2 and 140 mM, i.e. well-below and well-above the CMC (26.1 ± 0.8 mM, see the Supplementary Information (SI)).

It is important to stress here that, due to the crowding of the low-frequency region of the NMR spectrum, other authors [23] have chosen the homologue igepal-CA520 for their experiments.
Nevertheless, we decided to use the CO520, which is the surfactant typically employed in the aforementioned synthesis protocols [14,15,20–22].

(ii) The “ammonia microemulsions” were obtained by addition of liquor ammonia (2% v/v) to a 140 mM solution of igepal, i.e. the same proportion as in the syntheses published by some of the authors [20,21]. Then, proper amount of OAC, OAM or both were added, up to 2 mM.

(iii) The “water microemulsions” were obtained exactly as the ammonia ones, employing distilled water.

(iv) The “paramagnetic probe microemulsions” were prepared using a MnCl₂ water solution, either 0.3, or 3.0 or 30.0 mM.

(v) OAC-capped Bi₂S₃ nanoparticles were dissolved either in the absence or in the presence of igepal (140 mM) reverse micelles. OAC final concentration was ~2 mM. Then, the corresponding water microemulsion was prepared, either in the absence or in the presence of MnCl₂ (3.0 mM).

All of the samples were let to equilibrate overnight at room temperature, before performing the experiments.

Samples (700 µL) were loaded in 5.0 mm O.D. NMR tubes, and ¹H spectra were acquired with a Unity Inova 500NB high-resolution spectrometer (Agilent Technologies, California, USA) operating at a frequency of 500 MHz. Experiments were carried out at 300 K. Chemical shifts were referenced to the cyclohexane singlet (1.430 ppm with respect to TMS) before solvent suppression. The latter was achieved through application of the WET sequence [24,25], which uses a combination of shaped selective excitation (uburp shape was centered at 1.430 ppm with a width of ~100 Hz) and pulsed field gradients. ¹H spectra were acquired using a 6.7 µs pulse (90°), 1.5 s delay time, 2 s acquisition time and a spectral width of 6.7 kHz. ¹H-¹H correlation DQF-COSY experiment was recorded on igepal solution over the same spectral window using 2048 complex points and sampling each of the 512 increments with 64 scans. The same acquisition parameters have been applied, together with a mixing time of 100 ms, for the acquisition of TOCSY, and equal to either 50 or 100 ms for the NOESY spectrum.

3. Results and Discussion

3.1. Transmission Electron Microscopy

TEM analysis in the bright-field mode showed the formation of spheroidal (figure 1A) and rod-shaped (figure 1D) nanoparticles for the CoFe₂O₄ and Bi₂S₃ systems, respectively. Particle size distributions indicated a mean diameter of about 8 nm for CoFe₂O₄ nanoparticles; an average length of about 23 nm and a width of about 10 nm for Bi₂S₃ nanoparticles. The presence of the capping agents bound to the
nanoparticle surface kept them separated from each other, making the system ideal for the following phase of silica coating.

TEM analysis further allowed to verify the efficiency of the silica coating process. Figures 1B and 1C show the formation of spherical core/shell CoFe$_2$O$_4$/SiO$_2$ structures with an overall average size of 30 nm and a single magnetic core located at the sphere center. Due to the monodispersity, their assembling appeared to be in the form of hexagonal close packing. On the other hand, the Bi$_2$S$_3$ nanoparticles anisometry induced a deviation from the spherical shape of the core-shell system (figure 1E and 1F). Oval-shaped nanoparticles of about 50 nm in size were observed when a single nanoparticle was incorporated, while a more complex morphology was also visible if a multi-core composite was formed. In any case, for both the systems, neither free CoFe$_2$O$_4$/Bi$_2$S$_3$ nor empty SiO$_2$ nanoparticles were observed, confirming the high efficiency of the coating process.

![Fig. 1. TEM images in bright-field mode for capped (A) CoFe$_2$O$_4$ and (D) Bi$_2$S$_3$ nanoparticles. The images for the corresponding core-shell silica-coated composites are shown in (B,C) and (E,F), respectively, at different magnifications.](image)

3.2. Nuclear Magnetic Resonance

NMR has been proved to be a valuable tool to investigate both direct and reverse micelles, concerning either structure, dynamics and inter-molecular interactions [23,28–37]. In particular, in a recent study of the reaction mixture for the production of silica-coated nanoparticles [23], changes in the $^1$H-NMR chemical shift and line broadening were found to be very informative. Local environment
modifications of the different nuclei were monitored in different conditions starting from CHX solutions up to the formation of the microemulsion by adding water or liquor ammonia. The system’s evolution due to the ongoing reaction was also followed. Thus, this was the method of choice, aimed at clarifying the spontaneous behavior of igepal, OAC and OAM, both in CHX and CHX/water or CHX/ammonia microemulsions, before performing the experiments in the presence of the OAC-capped nanoparticles.

3.2.1. Igepal, OAC and OAM cyclohexane solutions

Figure 2 shows the $^1$H-NMR spectra of igepal (figure 2-A), OAC and OAM (figure 2-B) in CHX, together with a schematic representation of the molecules and the assignment of the most interesting resonances. The igepal spectrum is characterized by the presence of a myriad of signals due to the nonyl-tail in the low-frequency region (0-2 ppm), all the resonances due to the polyoxyethylenic protons in the mid-frequency region (3.4-4.2 ppm), and, in the high-frequency region (6.7-7.3 ppm), by the resonances of the aromatic protons. The latter are divided into two multiplets, one from the two nuclei located on the nonyl-tail side, the other due to the two nuclei on the polyoxyethylene one. Igepal was found to form reverse micelles in CHX with a CMC of 26 mM. The CMC for the reverse micelle of igepal in CHX was determined by following the $^1$H chemical shift variation as a function of igepal concentration, for the resonances labeled with $b$, $c$, $d$, $f$ and $g$ in figure 2-A (see figure S1). The chemical shift decreased with increasing the surfactant concentration, indicating an increased shielding from the main magnetic field. In terms of micelle formation, this is attributable to an increased local concentration of igepal due to the compactness of the micelle (see the SI for details). The observation of a single resonance for each of the proton groups, suggested that igepal molecules were in fast exchange between the reverse micelles and the solution’s bulk, regarding the NMR time scale.

As far as OAC and OAM, the triplet $b$ ($C\alpha H_2$) was the only resonance that did not overlap when they were mixed together, thus allowing monitoring the two molecules’ behavior separately. Both OAC and OAM have been found not to form any kind of micellar structure in CHX (up to 100 mM), but to be dispersed as monomers.
Fig. 2. The molecular structure of (A) igepal-CO520, (B-I) oleic acid and (B-II) oleylamine are schematically shown (the majority of the hydrogens are not explicitly reported for the sake of clarity), together with the corresponding $^1$H-NMR spectra in cyclohexane at 100, 2 and 2 mM, respectively. The most important resonances assignments are labeled with lower case letters. The spectral regions 6.7 - 7.3 and 3.4 - 4.2 ppm of igepal are zoomed in the insets. Some impurities present in the olylamine sample (not coming from the solvent, see the SI) are indicated by the asterisks.

An acid-base interaction was established when OAC and OAM were mixed together (1:1 molar ratio, 2 mM each), resulting in the formation of the corresponding ionic species (see the SI). At concentration higher than 8 mM, ion-pair amphiphiles aggregates formed as reported in the literature for similar systems [Fukuda H., Kawata K., Okuda H., Regen S.L., J. Am. Chem. Soc. 112, 1635-1637 (1990); Desando M.A., Lahajnar G., Sepe A., J. Colloid Interf. Sci. 345, 338-345 (2010)]. However, at the concentration used in the present investigation (2 mM each), the two molecules are mostly dispersed as monomers.

For the sake of brevity, we have reported only the main results, while the in-depth discussion and details are provided in the SI for the interested reader.

3.2.2. OAC/OAM in the presence of igepal

The behavior of OAC radically changed in the presence of igepal’s reverse micelles. The triplet due to the CαH$_2$ was found at a lower frequency (2.171 ppm) than that observed in the absence of igepal (2.265 ppm), thus being more shielded from the main magnetic field. In addition, the fine structure of
the multiplet at 5.325 changed dramatically (see figure S3). These observations strongly suggested that OAC spontaneously inserted into the igepal’s reverse micelles, in the experimental conditions employed (igepal 140 mM, OAC 2mM). The insertion was confirmed by additional experiments performed in the presence of igepal at different concentrations (2-140 mM). The OAC-CαH₂ triplet was found to shift towards lower frequency with increasing the igepal concentration. The data showed a dramatic slope variation at 27.4 mM, which is in very good agreement with the igepal CMC determined in this work (figure S4). In the case of OAM, similarly, the triplet due to the CαH₂ moved from 2.650 ppm to a lower frequency (2.634 ppm) in the presence of igepal above its CMC (140 mM), while it showed a comparable value (2.647 ppm) in the presence of igepal below its CMC (2 mM).

When both OAC and OAM (2 mM each) were present in the igepal’s reverse micelle dispersion, their acid-base equilibrium appeared to be further shifted towards the corresponding ionic forms (see the SI for details). This could be explained in terms of an increased probability for the two molecules to meet each other in solution and react, in the light of their tendency to insert in the igepal’s micelles.

In all of the investigated cases, the observation of only one resonance for each of the chemical groups, suggested that all these equilibria were fast, compared to the NMR time scale.

Further details are provided in the SI for the interested reader.

### 3.2.3. Liquor ammonia microemulsions

Figure 3-A shows the 3.4-4.2 ppm range of the ¹H-NMR spectra acquired on different liquor ammonia/igepal micro-emulsions.

The resonances from the polyoxyethylene chain of the igepal, as well as the band due to the liquor ammonia core of the reverse micro-emulsion at 3.955 ppm (figure 3-A(I)), were comparable to those reported in the literature [23].

The addition of OAM (figure 3-A(II)) did not significantly affect the position of the ammonia band, and only a slight broadening was observed. At first glance, this might be interpreted as the OAM not inserting in the microemulsion or, at least, not reaching its aqueous core. However, the triplet due to the CαH₂ group resulted to be shifted to a lower frequency (2.640 ppm) than observed for the OAM dispersion in CHX (2.650 ppm). This shift was comparable to that recorded for OAM in the presence of igepal’s reverse micelles (see subsection 3.2.2), thus suggesting OAM insertion.
Before giving a plausible explanation to this apparent contradiction, let us examine what happened upon OAC addition to the microemulsion, in the absence of OAM. The ammonia band shifted towards higher frequencies (3.987 ppm) and its width decreased (figure 3-A(III)). This was a clear indication that OAC inserted in the microemulsion with its acidic head group reacting with the ammonia and causing the band to move towards the value of the “neutral pH” water microemulsion [23]. The insertion of OAC was further supported by the position of the resonance due to the $\text{C}_\alpha\text{H}_2$ (2.145 ppm), which, again, was comparable to the value observed for the OAC inserted in the igepal’s reverse micelles (see subsection 3.2.2).

Now, if we simply compare the acid dissociation constants ($\text{p}K_a$ in water at 298K) of the molecules involved, we see that OAC ($\text{p}K_a \sim 5$) [39] is only a weak acid, but considering the presence of ammonia ($\text{p}K_a \sim 9$) [40] in the microemulsion core, OAC should be almost completely dissociated, explaining the NMR observations. On the other hand, OAM ($\text{p}K_a \sim 10.6$) [40] is expected to act as a very weak base,
providing a convincing explanation for the chemical shift of the CαH₂ indicating the insertion, while the ammonia band shows no significant variations.

Finally, when both OAC and OAM were added together to the ammonia/igepal microemulsion (figure 3-A(IV)), as expected, the position of their CαH₂ group (2.141 and 2.637 ppm, respectively) indicated the insertion towards the aqueous core. In addition, the ammonia band shifted to an even higher frequency (4.048 ppm) than observed after the addition of OAC only, with its line width further decreasing.

Overall, these results support the conclusions drawn on the basis of the experiments discussed hereinbefore. The tendency of OAC and OAM to insert in the igepal reverse micelles was not altered by the formation of the ammonia microemulsion. Their head group appeared to reach the inner core, where their acid-base equilibrium was established. A fast exchange between the inserted and the not-inserted state was evident for all species, on the basis of the observation of a single resonance for each magnetic group.

### 3.2.4. Water microemulsions

Complementary results were obtained from the experiments performed on water/igepal microemulsions.

Figure 3-B shows the 3.4 – 4.8 ppm range of the ¹H-NMR spectra acquired on different water/igepal micro-emulsions. The resonances from the polyoxyethylene chain of the igepal, as well as the band due to the aqueous core of the reverse micro-emulsion at 4.679 ppm (figure 3-B(I)), were in fairly good agreement with the literature [23].

The addition of either OAM (figure 3-B(II)) or OAC (figure 3-B(III)) caused the water band to broaden and shift to a lower frequency (4.604 and 4.624 ppm, respectively). Considering that the pH of the distilled water we employed was equal to 6.25, and taking into account the pKₐ of OAC and OAM reported hereinbefore, OAM was expected to act as a stronger base than OAC was as an acid. Indeed, this was reflected by a greater shift of the aqueous-core band caused by the former. The position of the CαH₂ of OAM and OAC was 2.643 and 2.188 ppm, respectively, i.e. lower than the value observed for the corresponding monomers dispersed in CHX.

Then, similarly to what was observed for the ammonia microemulsions, when both OAC and OAM were added together to the water/igepal microemulsion (figure 3-B(IV)), the water band shifted to a higher frequency (4.700 ppm) and its line-width decreased.
These results clearly showed the insertion of both OAC and OAM in the reverse microemulsion, and further supported their acid-base interaction taking place at the aqueous core. Again, a fast exchange between the inserted and the not-inserted state was inferred for all species, on the basis of the observation of a single resonance for each proton group.

For the sake of completeness, it has to be mentioned that, generally speaking, an in-depth interpretation of the chemical shift and line-width of the $^1$H-NMR aqueous band is extremely difficult and certainly beyond the scope of the present work. These two parameters strictly depend (at constant temperature and water content of the microemulsion) on the hydrogen-bonding network among the water molecules and the surrounding surfactants’ head groups, including proton exchanging phenomena and relaxation [23,41–44]. However, avoiding any overinterpretation of our data, it can be concluded that the local structure of the waters’ clusters and/or waters’ dynamics in the core of the microemulsion were altered by the addition of either OAC and/or OAM, to an extent that appeared to be correlated to the concentration of the ionic species formed, and ultimately depending, in turn, on the various acid-base equilibria involved.

### 3.2.5. Paramagnetic probe experiments

In order to further prove the insertion of both OAC and OAM in the microemulsion, several samples (water/igepal microemulsions) were prepared with increasing concentration of MnCl$_2$. The presence of the paramagnetic ion Mn$^{2+}$ in the water core, leads to a strong enhancement of both the longitudinal and transversal relaxation rate of the nuclei surrounding the water core. This effect is both distance and concentration dependent, thus, the resonances of the nuclei closest to the water core, will exhibit a dramatic broadening with increasing MnCl$_2$ concentration, while those located farther will be almost unaffected.

Figure 4 shows the 3.4 – 4.2 ppm range of the $^1$H-NMR spectra acquired on different water/igepal microemulsions prepared with a Mn$^{2+}$ concentration ranging from 0 to 30.0 mM. The broadening of the resonances attributed to the groups located closer to the hydroxyl head group (low-frequency side) was evident. On the contrary, the resonances due to the CH$_2$ groups located closer to the aromatic ring (high-frequency side), thus farther from the water core, resulted to be almost unaffected until the highest Mn$^{2+}$ concentration was reached. The water band showed the same broadening trend (not shown), until it almost completely disappeared under the spectral noise.

The same experiments were performed in the presence of OAC, resulting in the progressive broadening of the $^1$H resonance due to the CαH$_2$ group, until it completely disappeared under the spectral noise. On
the other hand, the resonances due to the protons adjacent to the unsaturation were not as dramatically affected. Exactly the same results were observed for both OAC and OAM, when they were added together to the microemulsion.

However, when OAM was added alone, in the case of the two samples with the highest Mn\(^{2+}\) concentration, a dark brown precipitate was found at the bottom of the NMR tube. Even with the lowest Mn\(^{2+}\) concentration, where the precipitate was not visible to the naked eye, the \(^1\)H-NMR spectrum suffered from a very poor magnetic-field homogeneity. Probably, due to the fact that, as said, the OAM head group acted as a base in the water/igepal microemulsion core, some water insoluble Mn-hydroxides were formed due to the pH rising and precipitated in the test-tube. Thus, even if paramagnetic resonance enhancement could not be monitored in the case of OAM, this was a clear indirect proof of its insertion into the microemulsion, even in the absence of OAC. In addition, the fact that no precipitation was observed when both OAC and OAM were present, further confirmed their acid-base interaction in the core of the microemulsion. OAC compensated for the pH increasing that OAM would cause, thus avoiding the precipitate formation.

![Fig. 4. The portion of the \(^1\)H-NMR spectrum comprising the resonances from the polyoxyethylene chain of the igepal-CO520 is shown. Spectra were collected on water (2% v/v) / igepal (140 mM) micro-emulsions in cyclohexane, with MnCl\(_2\) (I) 0, (II) 0.3, (III) 3.0 and (IV) 30.0 mM.](image)
Finally, chart 1 is provided to diagrammatically represent the main results achieved for all the equilibria discussed so far.

**Chart 1.** Diagrammatic representation of the main conclusions achieved in the absence of nanoparticles. (A) OAC is dispersed as neutral monomers in CHX, and do not form micellar aggregates. (B) Igepal CO-520 spontaneously forms micellar aggregates in CHX, with a CMC = 26 mM. (C) OAM is dispersed as neutral monomers in CHX, and do not form micellar aggregates. (D) In the presence of igepal’s reverse micelles, either OAC and/or OAM insert. Their head groups reach the hydrophilic core and, when both are present, their acid-base interaction is established. (E) Upon addition of either water or liquor ammonia, the core of the reverse micelle is filled with the aqueous solution: the micro-emulsion is formed. The head groups of either OAC and/or OAM are exposed to the aqueous core and, if both are present, their acid-base interaction is established.
3.2.6. Experiments with the OAC-capped Bi$_2$S$_3$ nanoparticles

When facing OAC-capped Bi$_2$S$_3$ nanoparticles, dynamics of the system was interestingly found to be rather different from all the previous cases. In the spectrum acquired on the nanoparticles dispersion, all the OAC’s resonances resulted to be somewhat broadened, when compared to the OAC monomers dispersion in the absence of nanoparticles. This is attributable to the fact that the OAC molecules present in the nanoparticle’s capping layer, as a whole, tumble slower than the monomers in solution [45].

The OAC resonance due to the CαH$_2$ group showed interesting spectral features suggesting an altered exchange rate between the monomers interacting with the nanoparticles in the capping layer, and those in the bulk solution. Figure 5 shows the 2.15-2.35 ppm range of the $^1$H-NMR spectrum acquired on different nanoparticles dispersions (the spectrum of OAC monomers in CHX is also shown for comparison). Unlike all previous cases, the resonance was not unique, but two distinct components were observed.

The spectrum (I) was collected on the capped nanoparticles, simply dissolved in CHX. A triplet was clearly resolved at 2.215 ppm, i.e. at a lower frequency than the corresponding resonance obtained for the monomers’ dispersion in CHX (2.265 ppm). However, a broader band was also observed on the high-frequency side of this triplet, centered at ~2.25 ppm. Whenever a chemical exchange takes place in solution, a well-resolved resonance should be expected for each of the two environments, if these two are sufficiently magnetically different (i.e. the corresponding difference in the resonance frequencies $\Delta\nu$ is sufficiently large), and if their exchange rate $k_{ex}$ is sufficiently slow. On the other hand, if $k_{ex} \gg \Delta\nu$, only the averaged resonance is observed, as it was in all the cases examined hereinbefore in the absence of nanoparticles. A rather complex variety of line shapes can be otherwise observed in-between, depending on the relative population of the two states and their intrinsic relaxation rates and scalar couplings [45]. Usually, going from fast to slow exchange, the averaged resonance broadens, eventually disappearing under the spectral noise, i.e. the so-called coalescence point. Then, with further decreasing the exchange rate, the two individual resonances progressively narrow and shift apart, until they become clearly distinguishable in the spectrum [45]. This actually seemed to be the situation pertaining to our results in the presence of nanoparticles. Due to nanoparticles preparation, OAC is present as its negatively charged conjugate-base (i.e. the oleate ion). When it is in the bound-state, carboxyl valence electrons are partially employed to bind the nanoparticle surface groups. On the other hand, when it is in unbound-state the head group electronic cloud should be more localized. Thus, the CαH$_2$ group is expected to be more shielded from the main
magnetic field in the unbound- than in the bound- state. The aforementioned relatively narrow triplet (figure 5-I) is thus attributable to the unbound-oleate, being this resonance component located at a lower frequency than the broad one, which is in turn assigned to the oleate in the bound-state. These attributions are also compatible with the different line-width of these two resonance components. The unbound-monomers are expected to be more mobile than the bound-ones, which being ‘anchored’ to the nanoparticle should tumble slower in solution. In other words, the overall rotational correlation time ($\tau_C$, i.e. the average time taken by the molecule to rotate by 1 radian) should be longer for the large molecular assembly constituted by the nanoparticle together with its amphiphiles capping layer, thus it is longer for the bound- than for the unbound- oleate molecules. Generally speaking, without going into a detailed analysis of all the possible contributions to spin relaxation, the line-width of a NMR resonance is proportional to the spin-spin relaxation rate, which is proportional to the overall rotational correlation time [45]. Indeed, the resonance component due the unbound-oleate is narrower than that due to the nanoparticle bound- molecules. The presence of these two states for the capping OAC, showed that, even if the capping layer was sufficiently stable to make the whole particle (i.e. the inorganic core plus the organic capping layer) hydrophobic and, thus, soluble in CHX, some monomers were able to detach from the nanoparticle surface.

When the capped nanoparticles were dispersed in CHX in the presence of igepal reverse micelles, the exchanging regime between the OAC bound and unbound state remained essentially the same. However, at the equilibrium, the difference in their relative population resulted to be significantly reduced. Figure 5-II, indeed, shows an almost unique broad resonance, where, nevertheless, the low-frequency triplet is still distinguishable. In addition, the igepal’s resonances due to the polyoxyethylenic units closest to the head group (not shown), were not insensitive to the presence of the nanoparticles, but resulted to be broadened. On the other hand, those attributed to the polyoxyethylene units closest to the aromatic ring were almost unaffected. These observations strongly suggested an interaction between the igepal and the nanoparticle’s surface through the hydrophilic head group.

Thus, from these results, it could be concluded that the detaching of OAC monomers from the nanoparticle surface is mainly due to their high affinity for the micelles of igepal, rather than a direct exchange with igepal molecules. Nevertheless, the igepal, whose monomers are always in equilibrium between the reverse micelles and the solution bulk, interacts with the partially naked nanoparticles and, at the equilibrium, the latter will be surrounded by a mixed organic layer (igepal/OAC) retaining the dispersibility in the nonpolar solvent.
Fig. 5. The portion of the $^1$H-NMR spectrum comprising the triplet of the methylenic group closest to the head group of the oleic acid is shown. Spectra were collected on a dispersion of OAC-$\text{Bi}_2\text{S}_3$ nanoparticles either in (I) cyclohexane and (II) a cyclohexane dispersion of igepal-C0520 140 mM, as well as after the addition of either (III) water or (IV) an aqueous solution of MnCl$_2$ 3.0 mM in the proper amount to form the micro-emulsion.

This “final” equilibrium state, in principle, might be also reached through the diffusion of the partially \textit{naked} and, thus, partially hydrophilic nanoparticle into the polar core of the reverse micelle. Alternatively, it could be originated from a direct ligand-exchange process simply due to the high excess of igepal, as invoked by some authors [13]. However, the present work showed a more complex scenario, where, comprising multiple equilibria, the driving force was the strong affinity of the capping ligand for the environment of the reverse micelles interior, rather than the igepal competing with OAC for nanoparticle binding.

The addition of water did not change the arrangement of this supramolecular assembly. In the resulting microemulsion, water molecules formed a sort of shell surrounding the nanoparticle. The interaction between the nanoparticle’s surface and the head group of the OAC was still effective, even if it resulted to be weakened. The intrinsic relaxation rate of the high frequency resonance component due to the nanoparticle bound OAC (figure 5-III) changed. The line-width decreased, indicating an increased
mobility (shortened $\tau_c$; decreased relaxation rate). Indeed, two triplets are now clearly distinguishable in the spectrum (figure 5-III). The addition of the paramagnetic probe Mn$^{2+}$ resulted in the broadening of both components (figure 5-IV), confirming OAC insertion into the reverse micro-emulsion, as well as the exchange between the inserted and the not-inserted molecules.

Chart 2 is finally provided, in order to diagrammatically represent the incorporation mechanism, as it emerged from the present work.

**Chart 2.** Diagrammatic representation of the mechanism for OAC-capped nanoparticles incorporation into the igepal reverse micro-emulsion. (A) When capped nanoparticles are dispersed in CHX, the OAC (i.e. the capping agent) is in equilibrium between the bound- and unbound- state. (B) In the presence of igepal’s reverse micelles, OAC high affinity for the latter results in the partial dissolution of the nanoparticles capping layer. (C) OAC in the nanoparticles capping layer is progressively replaced by igepal, until, at the equilibrium, the nanoparticles are mostly surrounded by the latter. (D) Finally, upon addition of the aqueous solution, the microemulsion is formed.

**4. Conclusions**

The present work provides new insights on the molecular interactions at the basis of the incorporation mechanism of colloidal hydrophobic-capped nanoparticles into the hydrophilic core of a reverse
microemulsion (igepal/water/cyclohexane). This is the very first stage of one of the most effective synthetic routes for the production of core-shell nano-architectures.

In order to elucidate this incorporation mechanism, a careful analysis of the chemical shift and multiplicity variations of selected $^1$H-NMR resonances was undertaken. The strategy was to firstly characterize the behavior of the ‘free’ capping agents in the presence of either the surfactants reverse micelles or the corresponding microemulsions and, successively, to verify the modifications induced by the presence of the nanoparticles. This approach, together with the employment of a paramagnetic probe as a relaxation enhancer, led to the recognition of fundamental details at a molecular level, such as the conformational transitions and insertion depth of capping agents upon interaction with the igepal micro-emulsion. This approach represents a novelty, when used in this context, and it has been extremely useful to gain a thorough understanding of the coexistent equilibria involved in such a complex system. The use of a paramagnetic probe, in particular, was fundamental, providing direct evidence of amphiphiles insertion into the microemulsion and thus cross-checking the interpretation of the other NMR results.

In the literature, only few models have been proposed to explain how a hydrophobic nanoparticle might be incorporated into the hydrophilic core of a micro-emulsion. Without any direct proof and based only upon phenomenological investigations, a mechanism was hypothesized [14] involving the tail-to-tail intercalation of oleic acid (OAC) in the nanoparticles’ capping layer with igepal CO520, and the subsequent interaction of the igepal’s polar head groups with water to form an external water shell. It is hardly convincing that igepal could direct its polar head towards a nonpolar solvent like cyclohexane and our results, indeed, rule out this model.

Much more reliable is the model suggesting the exchange of the hydrophobic capping agents by either the igepal [13, 16] and/or TEOS [13, 22], due to their higher affinity for binding the nanoparticle’s surface than the original capping ligands. However, our experimental data strongly suggest that the high affinity of the latter for the igepal’s micelles/micro-emulsions is the actual driving force of the incorporation mechanism, rather than a plain ligand-exchange process. The partially ‘naked’ nanoparticle would be unfavorably solvated by cyclohexane. The igepal, whose concentration largely exceeds that of the capping ligands, consequently substitutes the latter in the capping layer until, at the equilibrium, the nanoparticles are found to be surrounded by a mixed igepal-OAC shell.

Finally, upon addition of water or liquor ammonia, the micro-emulsion is formed, with the water molecules interacting both with the hydrophilic surface of the inorganic core of the nanoparticle, and
the polar heads of either OAC and igepal. This is the supramolecular assembly constituting the nano-reactor ready for the alkoxides addition and, thus, homogenous and size-tuned coating process.

In the light of the experiments presented, the intrinsic molecular interactions between the capping agents bound to the nanoparticle surface and the specific surfactant employed, play a key role. The present work, in addition, suggests how liquid-state NMR can be applied as a valuable tool in assisting synthesis design, allowing for different surfactants and/or ligands to be monitored using relatively low amounts of chemicals, before undertaking the synthesis trials.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://…

References


