Influence of molecular design on the morphology of nanoparticles formed from 1-alkyl-6-alkoxy-quinolinium cations and 4-sulfonatocalix[n]arenes

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In order to reveal the influence of the guest molecular structure, the interactions between 4-sulfonatocalix[n]arene (SCXn) cavatands (n = 4 or 6) and two series of quinolinium derivatives were studied in neutral aqueous solutions at 298 K. For this, the long alkyl chain of the quinoliniums was attached either to the heterocyclic nitrogen (C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{n}\)\(+\)) m = 10, 12, or 14) or to the oxygen located in position 6 of the aromatic system (C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{n}\)\(+\)) m = 8, 10, or 12). All the quinolinium derivatives self-assembled with SCXn into nanoparticles (NP), whose size, zeta potential and composition were determined over a large molar mixing ratio range. Isothermal titration calorimetry showed that host-guest binding assisted the formation of negatively charged NPs in exothermic processes. The enthalpy gain in these associations significantly increased with the lengthening of the 1-alkyl group but was insensitive to the size of the SCXn macrocycle. The morphology of NPs was studied by cryo-TEM method. C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{n}\)\(+\) organization with SCXn led to spherical NPs without regular inner structure. In contrast, C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{6}\)\(+\)-SCXn nanoaggregates usually had various shapes and the original morphologies exhibited lamellar domains with ~3 nm layer thickness. The different orientation of C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{6}\)\(+\) and C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{8}\)\(+\) in the cavatand was proposed to rationalize the morphological alterations.

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1. Introduction

Host–guest binding of simple molecular building blocks is a powerful tool to mediate the formation of a wide variety of supramolecular architectures [1]. The most frequently used hosts are cyclodextrins, which are valuable constituents of nanoparticle-based drug carrier systems [2]. They can modulate the self-organization of amphiphiles, and their composition of macromolecules, surface modification of nanoscale objects, and to create diverse nanostructures [8–9]. The length of the carbon chain and the degree of substitution significantly influenced the morphology and supramolecular structure of the nanoparticles fabricated from alkylated [1-cyclodextrins [10].

4-Sulfonatocalix[n]arenes (SCXn) can be more advantageously applied as hosts than cyclodextrins in the construction of self-assembled systems. This is related to the polyanionic character, pH-sensitivity, larger solubility and conformational flexibility of the SCXn macrocycles. Additionally, their substantial binding affinity towards amphiphilic guests facilitate the further association into nanoparticles [11], vesicles [12–14], supramolecular micelles [15–17], or stimuli-responsive nanostructures [18–20]. A recent paper provides excellent overview on the advances in the recognition and assembly properties of this type of cavitands [21]. The structural motifs of SCXn complexes have been intensively examined in the solid state [22,23], but the relationship between the molecular structure of SCXn-containing supramolecular amphiphiles and the properties of their nanoparticles produced in solution is still not fully understood. It cannot be predicted how the characteristics of the constituents influence the size, shape and inner structure of the nanospecies created by spontaneous self-organization in water.

To find general trends, the main goal of the present studies was to reveal how the molecular structure variation affects the enthalpy of association and the morphology of the nanoparticles yielded by simple mixing of the components. We focused on the SCXn-induced aggregation of a new family of amphiphiles possessing a functionalized quinolinium head group. Two series of quinolinium derivatives were synthesized in which the long alkyl chain was attached either to the heterocyclic nitrogen (C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{n}\)\(+\)) m = 10, 12, or 14) or to the oxygen located in position 6 of the aromatic system (C\(_{10}\)C\(_{6}\)O\(_{2}\)Q\(_{n}\)\(+\)) m = 8, 10, or 12). Formulas of the employed compounds are presented in Scheme 1. Our previous study demonstrated that the homologues comprising...
shorter aliphatic groups produced only 1:1 complexes with 4-sulfonatocalix[4 or 6]arenes in enthalpy driven processes and larger aggregates were not generated [24]. Additionally, NMR results showed different incorporations of the aliphatic group into SCX4: four carbons of the N-alkyl chain were located inside the macrocycle, while the alkoxy chain entirely protruded from the SCX4 cavity. Now, we intend to unravel how such kinds of differences alter the internal structure of self-assembled nanoparticles. The variation of the molecular structure of the constituents is an appealing way to tune the shape and internal geometric arrangements of nanosized aggregates. The control of not only the size but also the morphology of nano-objects would be highly beneficial in many potential applications. Hence, information on the relationship between chemical structure and the ordering of the building blocks in the course of self-organization is of crucial importance for the rational design of tailor-made products.

2. Materials and methods

2.1. Materials

4-Sulfonatocalix[4]arene (SCX4) and 4-sulfonatocalix[6]arene (SCX6) were purchased from Acros Organics and used after drying under vacuum at 343 K overnight. Double distilled water served as solvent and the pH of the SCXn solutions was adjusted to 7 using the minimum volume of concentrated NaOH. The different 1-alkyl-6-alkoxy-quinolinium salts were synthesized and characterized by 1H NMR spectroscopy as described in the Supporting Information. CmC1OQ+ cations were used as Br− salts, whereas CmC1OQ+− ions had −1 counterions. The preparation of 1-tetradecyl-6-methoxy-quinolinium bromide (C4C1OQ+Br−) has been reported [17].

2.2. Sample preparation

Nanoparticles (NP) were generated by mixing the appropriate amounts of 2 mM or 4 mM 1-alkyl-6-alkoxy-quinolinium and 1 mM SCXn (pH 7) solutions under stirring at 150 rpm. Because of the low solubility, the 1 mM stock solutions of the C1C1OQ+−1 and C10C1OQ+−1 compounds were gentle heated before each use. SCXn concentration was kept constant (0.1 mM) unless otherwise noted. All experiments were conducted at neutral pH, where according to the reported deprotonation constants [25], SCX4 and SCX6 contained either one or two phenolate groups, leading to a 5-fold or 8-fold negatively charged SCX4 and SCX6 anions, respectively.

2.3. Instrumentation

1H NMR spectra were recorded in deuterated DMSO on a Bruker Avance II 400 MHz NMR spectrometer. The absorption spectra were obtained on an Agilent Technologies Cary60 spectrophotometer. Particle size was determined by dynamic light scattering on a Zetasizer Nano-ZS (Malvern Instrument) equipped with a He−Ne laser (λ = 633 nm, scattering angle 173°) at 296 K. Each measurement was the average of 12 runs of 10 s. Data were analyzed with the software developed by the manufacturer using a distribution analysis (General Purpose analysis). The mean diameter of the NPs was calculated on the basis of number distribution. Experiments were repeated at least twice. NPs were separated from the liquid phase by an ultracentrifuge from Beckman Coulter (Optima Max-XP, type TLA 110 rotor). Total carbon analyses were performed on a Shimadzu TOC-L CSN instrument, which was calibrated by a potassium hydrogen phthalate solution in ultrapure water (2.125 g dm−3 corresponding to 1000 mg C dm−3). ITC measurements were carried out with a MicroCal VP-ITC microcalorimeter at 298 K. Quinolinium solutions were injected from the computer controlled microsyringe at an interval of 180 s into the cell (volume = 1.4569 mL) containing 0.1 mM SCXn solution at pH 7, while stirring at 450 rpm. Cryogenic transmission electron microscopy images were taken on an UltraScan 2 k × 2 k CCD camera (Gatan, USA), using a LaB6, JEOL JEM 2100 (JEOL, Japan) cryo-microscope operating at 200 kV with a JEOL low dose system (Minimum Dose System, MDS) to protect the thin ice film from any irradiation before imaging and to reduce the irradiation during the image capture. The images were recorded at 93 K and digitally corrected using the ImageJ software. The samples were prepared as previously reported [11].

3. Results

3.1. Micelle formation

Before the study of the association with SCXn, the enthalpy change upon dilution of 1-alkyl-6-methoxy-quinoline aqueous solutions was examined by isothermal titration calorimetry (ITC). The solubility of 1-alkyl-6-alkoxy-quinolinium iodides (CmC1OQ+I−) was low at 298 K. For example, it was lower than 4 mM for C10C1OQ+I−. The addition of close to saturated C10C1OQ+I− solutions to water brought about negligible enthalpy variation indicating the insignificant ion association of these amphiphiles even at the highest concentration reached in water at 298 K. No micelles were formed at this temperature.

In contrast, larger solubility could be attained for 1-alkyl-6-methoxy-quinolinium bromides (CmC1OQ+Br−). Fig. S1 presents the results of ITC experiments for the injection of 13.8, 14.8, and 88.6 mM solutions of tetradecyl, dodecyl-, and decyl homologues into water, respectively. Endothermic dilutions were always found and the enthalpograms demonstrated the association into micelles in the titrant solutions. In the initial stage of titration, the micelles fully dissociated because the concentration in the final solution remained below the critical micelle concentration (cmc). The small incipient increase of ΔH with concentration for C10C1OQ+Br− and C10C1OQ+I− originated from the contribution of the electrostatic interactions among ions to the dilution heat [26]. The sharp diminution of ΔH implied that cmc was reached in the titrant solution and as a consequence, the heat evolution in this second concentration domain was due to the dilution of micellar solutions. The location of the inflexion point, which was determined from the minimum of the first derivative of the enthalpograms, corresponded to the cmc [27]. ITC measurements at 298 K provided

Scheme 1. Chemical structure of the components used in the present study.
0.66 mM, 2.65 mM, and 11.6 mM values for the cmc of C_{12}C_{10}OQ'^{+}Br^−, C_{12}C_{8}OQ'^{−}Br^−, and C_{12}C_{16}OQ'^{−}Br^−, respectively. The heat release upon dilution indicated that the association of these amphiphiles to micelles was enthalpically favored. The lengthening of the aliphatic chain decreased the ΔH of micellization in the series of ΔH(C_{14}C_{m}OQ'^{+}) = −5.7 kJ mol^−1 > ΔH(C_{12}C_{m}OQ'^{+}) = −7.4 kJ mol^−1 > ΔH(C_{10}C_{m}OQ'^{+}) = −11.9 kJ mol^−1.

3.2. Calorimetric study of the self-assembly with SCXn

To ensure that no micelle formation took place in the titrant solution, 1-alkyl-6-alkoxy-quinolinium concentration was always kept below the cmc in the course of ITC measurements of the self-organization with SCXn at 298 K. The enthalpograms were corrected by the dilution heat.

When SCX4 was titrated with C_{12}C_{8}OQ'^{+}, the enthalpogram showed a peculiar shape (Fig. 1A). After the substantial incipient enthalpy (ΔH) release, [ΔH] reached a minimum at [C_{12}C_{8}OQ'^{+}]/[SCX4] = 1.4 mixing ratio, and then, increased attaining a plateau. Upon further increase of C_{12}C_{8}OQ'^{+} concentration, [ΔH] gradually vanished in the 5.5 < [C_{12}C_{8}OQ'^{+}]/[SCX4] < 8.0 range. These trends indicate two major association processes. The initial enthalpy gain is attributed to complex formation because the first part of the experimental data can be fitted assuming 1:1 host-guest binding (Fig. 1B). The nonlinear least-squares analysis provided an association constant of K around 1.2 × 10^5 M^−1 assuming 1:1 host-guest binding (Fig. 1B). The nonlinear least-squares analysis provided an association constant of K around 1.2 × 10^5 M^−1 > 1:1 complexation model.

The complex and NP formations are less separated when C_{12}C_{m}OQ'^{+} is added to SCX4 solution instead of C_{12}C_{8}OQ'^{+} (Fig. S2) because the increase of the aliphatic chain length leads to somewhat more exothermic self-organization into NPs at lower quinolinium concentration. The low solubility of C_{12}C_{m}OQ'^{+} did not allow calorimetric experiments, but NPs production was found (vide infra).

Fig. 2 presents the enthalpograms for the titration of SCX4 with various C_{12}C_{m}OQ'^{+} derivatives. The lengthening of the aliphatic chain linked to the heterocyclic nitrogen brought about similar behavior to that found for C_{12}C_{8}OQ'^{+}. In the case of the quinolinium derivatives possessing shorter alkyl substituent, the association to complex and NP were more separated (Fig. 2A). For the homologues with longer carbon chain, the association into NPs started at lower [C_{m}C_{10}OQ'^{+}]/[SCX4] mixing ratio and occurred in a wider range. The complexation was almost completely masked by NP formation for C_{12}C_{10}OQ'^{+} (Fig. 2C). The ΔH values at the plateau, which corresponded to the enthalpy change upon NP production, became more negative with the increase of the chain length. When C_{10}C_{10}OQ'^{+} was used as a titrant, the initial part of the enthalpogram could be fitted well assuming 1:1 binding and an association constant (K) of around 1.5 × 10^5 M^−1 was obtained. This result fits to the tendency found for SCX4 complex formation of the smaller C_{m}C_{10}OQ'^{+} cations [24] (m ≤ 8) where K varied from 4.6 × 10^5 M^−1 to 2.1 × 10^7 M^−1 upon increase of m from 1 to 8.

The larger SCX6 macrocycle induced similar association processes with the different 1-alkyl-6-alkoxy-quinoliniums (Figs. S3 and S4). The ΔH values of NP formations are summarized in Table 1. The alteration of the cavity size and the length of the alkoxy substituent of C_{12}C_{m}OQ'^{+} slightly influenced the exothermicity of NPs formation. In contrast, about 4.4- and 2.5-fold enthalpy gain enhancements were observed when the N-alkyl moiety of C_{m}C_{10}OQ'^{+} was varied from decyl to tetradecyl in the case of association with SCX4 and SCX6, respectively. In addition, longer alkyl group was necessary for NP formation when the hydrophobic carbon chain was attached to the heterocyclic nitrogen of the quinolinium ring instead of the oxygen at position 6. Our previous study demonstrated that both the quinolinium ring and the four first CH_{2} groups linked to the heterocyclic nitrogen of C_{12}C_{m}OQ'^{+} guests are incorporated in SCX4 macrocycle upon 1:1 complexation [24]. In the case of C_{12}C_{8}OQ'^{+}−SCX4, the fraction of the N-octyl moiety located outside the host is too short to induce NP formation. In contrast,
C14C1OQ+ -SCX4 complex initiates NP production in substantially exothermic process because the alkyl group connected via oxygen to the quinolinium ring protrudes from the SCX4 cavity [24]. Such a conformation permits efficient ordering of the alkyl chains and π-π interaction among the aromatic systems within the NPs. These effects also lead to much more negative ΔH for C1C10OQ+ -SCX4 NPs than for C1C1OQ+ -SCX4 NPs.

Surprisingly, despite its relatively short octyl group, C18C1OQ+ aggregates both with SCX4 and with SCX6 into NP in more exothermic processes (Table 1) than 1-methyl-3-tetradecyl-imidazolium (C14mim+), which has a much longer hydrophobic tail. The enthalpy change upon formation of C14mim+-SCX4 and C14mim+-SCX6 NPs were found to be ΔH = −10.9 and −10.8 kJ mol⁻¹ in water at 298 K, respectively [16]. The substantial ΔH difference for the NPs of C18C1OQ+ and C14mim+ suggests that the strength of the binding to the host and the interactions among the cationic headgroups of the amphiphiles significantly contribute to the stability of NPs. The more extended aromatic character of the quinolinium compared with imidazolium allows stronger π-π interaction with the benzene rings of SCXn as indicated by the more exothermic encapsulation of 6-methoxy-1-methylquinolinium [24] (C1C8OQ+) in SCX4 (ΔH = −38.7 kJ mol⁻¹) and in SCX6 (ΔH = −32.1 kJ mol⁻¹) than the corresponding values obtained for 1,3-dimethylimidazolium inclusion [28] in SCX4 (ΔH = −29.2 kJ mol⁻¹) and in SCX6 (ΔH = −29.5 kJ mol⁻¹) in water at 298 K. In addition, the positive charge of the headgroup is more delocalized in quinolinium than in imidazolium. As a consequence, the repulsion among the cationic moieties is weaker within the NPs of C1C10mim+ -SCXn types than within C14mim+-SCXn NPs.

### Table 1

| Host   | Guest      | Mixing ratio [C14C1OQ+]/[SCX4] | ΔH[kJ mol⁻¹] |
|--------|------------|--------------------------------|--------------|
| SCX4   | C10C1OQ+   | m = 8                          | −14.9        |
|        | C10C1OQ+   | m = 10                         | −16.7        |
|        | C10C1OQ+   | m = 12                         | −15.2        |
|        | C10C1OQ+   | m = 14                         | −20.2        |
| SCX6   | C10C1OQ+   | b                              | −4.6         |
|        | C10C1OQ+   | a                              | −15.1        |

*Measurement cannot be performed because of the low solubility of the guest.

3.3. Dynamic light scattering and zeta potential

To gain information on the size and charge of NPs, dynamic light scattering and zeta potential measurements were performed. Fig. 3 displays the results achieved upon addition of C14C1OQ+ to SCX4 solutions. Negatively charged NPs were already produced at equimolar mixing ratio. Aggregation to larger particles took place when [C14C1OQ+]/[SCX4] ≥ 11 mixing ratio, whereas coalescence occurred only at [C14C1OQ+]/[SCX4] ≥ 12. The zeta potential progressively approached to zero when the relative amount of C14C1OQ+ was raised in the solution. Positively charged NPs with a diameter larger than 350 nm appeared only at substantial (17–20-fold) quinolinium excess, but they were unstable and coalesced in less than 1 h. A similar behavior was observed with SCX6 and size and zeta potential of NPs formed between SCX6 and C18C1OQ+ are given in Fig. S8.

With C1C10OQ+ homologues, NP formation started already in the case of the octyl derivative and the characteristics of C1C1OQ+ -SCX4 NPs (Fig. S9) were similar to those of C10C1OQ+ -SCX4 NPs. Negative particles were formed up to [C10C1OQ+] /[SCX4] = 8 mixing ratio. On further shortening of the N-alkyl substituent to decyl led to different behavior (Fig. 4). Namely, negatively charged NPs were formed up to [C10C1OQ+] /[SCX4] = 11 mixing ratio, whereas coalescence occurred only at [C10C1OQ+] /[SCX4] ≥ 12. The zeta potential progressively approached to zero when the relative amount of C10C1OQ+ was raised in the solution. Positively charged NPs with a diameter larger than 350 nm appeared only at substantial (17–20-fold) quinolinium excess, but they were unstable and coalesced in less than 1 h. A similar behavior was observed with SCX6 and size and zeta potential of NPs formed between SCX6 and C18C1OQ+ are given in Fig. S8.

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the other hand, the variation of mean diameter and zeta potential of C_{10}C_{10}OQ^{+}–SCXn NPs increased as a difference between the total concentrations and the concentrations in the liquid phase. After the separation of NPs by ultracentrifugation, the supernatants were analyzed by total carbon content measurements and the quinolinium amount was obtained by spectrophotometry [111]. Fig. 5 presents the C_{m}C_{10}OQ^{+} :SCXn molar ratio in the NPs as a function of the mixing ratio of the constituents. The C_{4}C_{10}OQ^{+} :SCX4 stoichiometry barely varied within the particles and the mean value was 5.5. The SCX4 percentage in the NPs rose up to a mixing ratio of 5. Above this value >95% of SCX4 was included in the NPs. On the other hand, >95% of C_{4}C_{10}OQ^{+} was included in the NPs until the mixing ratio of 5, and then the excess amount of C_{4}C_{10}OQ^{+} unbound in NPs increased. Anyhow, the C_{4}C_{10}OQ^{+} concentration in the supernatant remained below 1% even at high mixing ratios. The almost constant chemical composition of NPs (C_{4}C_{10}OQ^{+} :SCX4 = 5.5 and C_{4}C_{10}OQ^{+} :SCX6 = 7.2) was close to the charge compensation between the quinolinium cations and the polyanionic macrocycle. At pH 7, SCX4 had 5, whereas SCX6 had 8 negative charges because not only were the sulfonate groups deprotonated but also one or two phenolic OH group(s) released proton negative charges because not only were the sulfonate groups deprotonated but also one or two phenolic OH group(s) released proton upon association (Table 1). The nearly axial orientation of the alkyl chain in C_{m}C_{10}OQ^{+}–SCXn NPs is also possible because the sodium cation is a SCXn decorated with as much C_{10}C_{10}OQ^{+} or C_{10}C_{12}OQ^{+} as necessary to ensure charge compensation.

3.4. Stoichiometry of the constituents in the NPs

The relative amounts of the constituents in the NPs were determined as a difference between the total concentrations and the concentrations in the liquid phase. After the separation of NPs by ultracentrifugation, the supernatants were analyzed by total carbon content measurements and the quinolinium amount was obtained by spectrophotometry [111]. Fig. 5 presents the C_{m}C_{10}OQ^{+} :SCXn molar ratio in the NPs as a function of the mixing ratio of the constituents. The C_{4}C_{10}OQ^{+} :SCX4 stoichiometry barely varied within the particles and the mean value was 5.5. The SCX4 percentage in the NPs rose up to a mixing ratio of 5. Above this value >95% of SCX4 was included in the NPs. On the other hand, >95% of C_{4}C_{10}OQ^{+} was included in the NPs until the mixing ratio of 5, and then the excess amount of C_{4}C_{10}OQ^{+} unbound in NPs increased. Anyhow, the C_{4}C_{10}OQ^{+} concentration in the supernatant remained below 1% even at high mixing ratios. The almost constant chemical composition of NPs (C_{4}C_{10}OQ^{+} :SCX4 = 5.5 and C_{4}C_{10}OQ^{+} :SCX6 = 7.2) was close to the charge compensation between the quinolinium cations and the polyanionic macrocycle. At pH 7, SCX4 had 5, whereas SCX6 had 8 negative charges because not only were the sulfonate groups deprotonated but also one or two phenolic OH group(s) released proton negative charges because not only were the sulfonate groups deprotonated but also one or two phenolic OH group(s) released proton upon association (Table 1). The nearly axial orientation of the alkyl chain in C_{m}C_{10}OQ^{+}–SCXn NPs is also possible because the sodium cation is a SCXn decorated with as much C_{10}C_{10}OQ^{+} or C_{10}C_{12}OQ^{+} as necessary to ensure charge compensation.

3.5. Morphology of nanoparticles

To reveal the shape and internal structure of NPs in their aqueous environment, cryogenic transmission electron microscopy (cryo-TEM) images were recorded after mixing the components at 296 K. Fig. 6 shows representative results for negatively and positively charged NPs taken after adding C_{4}C_{10}OQ^{+} to SCX4 solution in molar ratio of 5 and 9, respectively. Both types of NPs appeared as well-separated uniformly dense spheres, as previously reported [15–17] for negative C_{4}C_{10}OQ^{+}–SCXn NPs. The positive C_{4}C_{10}OQ^{+}–SCXn NPs were also spherical, but some of them appeared as stacked circle planes (Fig. S13C, such structures have been reported for the NPs composed of C_{10}mim^{+} and SCX6 [111]). Cryo-TEM images showed comparable particle size to the mean diameter determined by DLS measurements (Table S1). Dense spherical nanoparticles were also observed for other C_{m}C_{10}OQ^{+}–SCXn NPs and C_{4}C_{6}OQ^{+}–SCXn NPs at different molar mixing ratios (Fig. S14).

Interestingly, the positively charged C_{4}C_{10}OQ^{+}–SCXn and C_{4}C_{12}OQ^{+}–SCXn NPs provided entirely different images (Figs. 7, 8, and Figs. S15–S17). They had non-spherical shape and internal structures showing equidistant lines about 3 nm apart in various areas of the nanoscale objects indicating periodic spacing of molecular layers. This regular spacing could correspond to the size of one self-assembled unit that is a SCXn decorated with as much C_{10}C_{10}OQ^{+} or C_{10}C_{12}OQ^{+} as necessary to ensure charge compensation.

The C_{4}C_{10}OQ^{+}–SCX4 NPs showed very well-defined structures with periodic spacing of molecular layers. Although the shape of NPs was not spherical, angular edges were noticed for most of them (Fig. S15). Many C_{4}C_{10}OQ^{+}–SCX6 NPs had a barrel-like appearance. They were partly aggregated and exhibited parallel lattice fringes (Fig. S16). The positively charged C_{4}C_{12}OQ^{+}–SCX4 NPs were inhomogeneous with a variety of profiles. A few of them exhibited empty space inside and nonuniform density. Aggregation and patterns like a fingerprint were also noticed (Fig. S17). In the case of C_{4}C_{12}OQ^{+}–SCX6, different types of organizations also coexisted. Nanoarchitectures of different shape, size and...
contrast were observed. Some had a well-defined contour; others had a more diffuse border. The internal structure extended to different domains developing fingerprint-like pattern (Fig. S18).

4. Discussion

The location and length of the alkyl chain on the quinolinium are the determining parameters for the shape and internal structures of NPs. Unstructured and spherical NPs appear for \( C_mC_1OQ^+\text{-SCX}_n \), whereas structured architectures are produced for \( C_1C_mOQ^+\text{-SCX}_n \). Among the different nano-assemblies formed with SCXn \([11,16-18]\), the ones obtained with \( C_1C_mOQ^+ \) \((m>8)\) were the first that led to non-spherical but structured aggregates. These structured morphologies remind the ones formed by nanoparticulate ordered mesophases of lipids, cubosomes or hexosomes \([29,30]\). In the present study, the nanoparticulate assemblies correspond to \( C_1C_mOQ^+\text{-SCX}_n \) associates with precise stoichiometries (vide supra) and not to \( C_1C_mOQ^+ \) aqueous mesophases. Putaux and coworkers observed the self-assembly of \( \beta \)-cyclodextrins grafted with octyl or dodecyl groups into hexosomes \([10]\). When the total degree of substitution (TDS) of the cyclodextrin cavitand was >5, barrel-like assemblies with columnar inverse hexagonal structure (hexosomes) were produced, whereas spherical NPs with a multibilayer structure were created at TDS < 5.

The morphology differences between \( C_mC_1OQ^+\text{-SCX}_n \) and \( C_1C_mOQ^+\text{-SCX}_n \) may be attributed to conformational changes of SCXn upon association with one or the other type of amphiphile. Indeed, SCX6 is known to have a high molecular flexibility and can adopt several different conformations among which the double partial cone structure is the most probable \([31,32]\). SCX4 is more rigid and exist predominantly in cone conformation which undergoes ring inversion \([33]\). Despite the differences in the conformational mobility of SCX4 and SCX6, the NPs of both cavitands undergo a change from disordered nano-assemblies to structured aggregates when the nature of their amphiphile constituents is altered. This suggests that the structural features of NPs are probably controlled by the modification of the orientation of the amphiphilic guest in the host cavity and the conformational changes of the SCXn macrocycles play a less important role. The morphology of NPs composed of 1-alkyl-6-methoxy-quinolium-SCXn supramolecular amphiphile units also alters with the molecular structure of the constituents. Although each SCXn is surrounded by as much cationic amphiphiles as their total anionic charge (5 for SCX4 or 8 for SCX6), the organization of within these building blocks strongly depends on the location of the alkyl chain on the quinolinium moiety.
When $\text{C}_m\text{C}_1\text{OQ}^+$ cations bind to SCX$n$, a few of the methylene groups close to the heterocyclic nitrogen are situated within the macrocycle whereas the alkyl substituents of other quinoliniums protrude from the cavity (Scheme 2A). Such ordering leads to looser interaction among the aliphatic chains which impedes the alignment of the $\text{C}_m\text{C}_1\text{OQ}^+$-SCX$n$ units into layers. Therefore, the formation of spherical NPs without any regular inner structure becomes preferable.

In contrast, the alkoxy chains are oriented outward from the host macrocycle within $\text{C}_1\text{C}_m\text{OQ}^+$ segments which allows tighter interactions (Scheme 2B). Hence, these building elements can be arranged into lamellar structures. The $\text{C}_1\text{C}_m\text{OQ}^+$-SCX$n$ associates are the supramolecular analogues of $\beta$-cyclodextrin grafted with alkyl groups ($\beta$-CD-$\text{C}_n$). In the former case, 5 or 8 alkoxy-substituted quinolinium cations are linked by electrostatic and hydrophobic interactions to SCX4 and SCX6, respectively, while 7 aliphatic chains are covalently attached to each macrocycle in $\beta$-CD-$\text{C}_n$. Due to the closely related structures of their building blocks, $\text{C}_1\text{C}_m\text{OQ}^+$-SCX4 and $\text{C}_1\text{C}_m\text{OQ}^+$-SCX6 NPs exhibited similar but less well defined morphology to those composed of $\beta$-CD-$\text{C}_n$ molecules [10]. When SCX4 was replaced with SCX6, ultrastructuring and barrel-like arrangements were more clearly observed for $\text{C}_1\text{C}_m\text{OQ}^+$-SCX6 NPs. In some cases, crystal structures are organized in the three-dimensional space with planes intersecting at about 120° for NPs comprising SCX4 (Fig. 7A) or SCX6 (Fig. S18). This may indicate local hexagonal structure. We can infer from our cryo-TEM results that the self-assembly of $\text{C}_1\text{C}_m\text{OQ}^+$ with SCX$n$ brings about nanoparticulate ordered mesophases. These kinds of structures have attracted considerable attention because of their large surface area and ability to solubilize both hydrophilic and hydrophobic compounds.

5. Conclusions

The association features of $\text{C}_1\text{C}_m\text{OQ}^+$ and $\text{C}_m\text{C}_1\text{OQ}^+$ considerably differ both in the presence and absence of SCX$n$ cavittands. Only the latter type of amphiphiles is able to produce micelles. The micellization of $\text{C}_m\text{C}_1\text{OQ}^+$-$\text{Br}^-$ homologues is exothermic if the 1-alkyl substituent is composed of at least 10 carbon atoms. The cmc diminishes about 17-fold when the 1-decyl substituent is changed to 1-tetradeacyl. $\text{C}_1\text{C}_m\text{OQ}^+$ cations do not associate because their iodide salt has low solubility. However, even the octyl homologue of $\text{C}_1\text{C}_m\text{OQ}^+$ is capable of assembly with SCX$n$ into nanoparticles because of the substantial enthalpy diminution of this process. The nearly axial outward orientation of the alkoxy group from the host interior facilitates the binding of as many $\text{C}_1\text{C}_m\text{OQ}^+$ cations as needed for the compensation of the SCX$n$ charge. The building blocks produced thereby self-organize into mesophase domains, which are incorporated into NPs of irregular shape. In contrast, a part of the aliphatic moiety of SCX$n$-linked $\text{C}_m\text{C}_1\text{OQ}^+$ amphiphiles is included in the host. This impedes the ordering of the methylene chains into layers and the formation of spherical NPs without any regular inner structure becomes preferable. Because of the unfavorable direction of the alkyl group in $\text{C}_m\text{C}_1\text{OQ}^+$-SCX$n$, longer carbon chain is required for the aggregation into NPs than in the case of $\text{C}_1\text{C}_m\text{OQ}^+$-SCX$n$ and the enthalpy change upon association alters to a larger extent with the increase of the number of methylene groups. The exothermicity and the mode of aggregation into NPs depends, to a great extent, on the orientation of the hydrophobic tail within the supramolecular amphiphile, but alteration of the macrocycle from SCX4 to SCX6 causes a minor effect. The morphologies obtained with $\text{C}_1\text{C}_m\text{OQ}^+$ constituents correspond to nanoparticulate ordered mesophases. Such structures attract widespread interest owing to their versatile potential applications.

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Declaration of competing interest

None.
Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molliq.2019.111656.

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