

Nanoassemblies from Vitamin C Derivatives

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ABSTRACT

Vitamin C is one of the most effective natural antioxidants. However, due to its poor solubility in non-aqueous environments, its use is restricted to water-based systems. In order to extend its advantages to lipophilic media, vitamin C must be converted into amphiphilic derivatives.

Two new vitamin C-based surfactants were synthesized: a bolaform derivative (1,12-diascorbyl dodecanedioate, BOLA12), and a double chain product (2-octyl-dodecanoyl-6-O-ascorbic acid, 8ASC10).

Once dispersed in water above 0.5% w/w, BOLA12 forms hollow nanotubes that transform into clear micellar solutions on heating. 8ASC10 is poorly soluble in aqueous media, but it dissolves easily in cyclohexane where it forms true organogels.

Cryo-TEM, XRD, SAXS, conductivity, viscosity and DSC experiments were performed in order to study the aggregation properties and morphology of these nanoassemblies.

Keywords: vitamin C, ascorbic acid, antioxidant, nanoparticles, organogel

1 INTRODUCTION

Vitamin C is one of the most powerful natural antioxidants. It inhibits radical-initiated lipid peroxidation, and is supposed to oppose the activity of free radicals involved in the development or exacerbation of several pathologies such as Alzheimer's disease, ischemia-reperfusion disturbances, rheumatism, inflammatory disorders, cancer and so on [1]. However ascorbic acid is hydrophilic and very poorly soluble in organic media, and therefore cannot penetrate natural membranes. In order to extend its anti-radical activity to lipophilic environments, vitamin C has to be transformed into amphiphilic derivatives, that offer the same antioxidant activity of the parent molecule, and produce self-assembled supramolecular aggregates in water- or in oil-based dispersions. In such conditions, the

solubility, stability and availability of hydrophobic chemicals can be greatly enhanced [2]. We have already reported the synthesis and physico-chemical characterization of single-chain vitamin C-based surfactants [3]. More recently we synthesized a bolaform surfactant (BOLA12) that contains two ascorbic acid headgroups linked by an alkyl chain [4]. This product dissolves in water, and – depending on the concentration and temperature – produces cylindrical nanoassemblies. The presence of two aliphatic chain in another derivative (8ASC10) enhances the solubility in organic solvents and decreases dramatically its solubility in water. This novel product forms stable organogels in the presence of organic solvents such as cyclohexane. The properties of BOLA12 and 8ASC10 are presented in this work.

2 MATERIALS AND METHODS

2.1 Synthesis

All reagents (analytical grade) were purchased from Fluka (Milan, Italy) and used without further purification. Isocarb20[®] was a gift from Sasol GmbH (Germany). Nitrogen was bubbled through 150 mL of 95% sulfuric acid, at room temperature and under magnetic stirring for 30 min. 8.7 g of 1,12-dodecanedioic acid (for BOLA12), or 15 g of dry Isocarb20[®] (for 8ASC10) were added and completely dissolved prior to the addition of 20.2 g (for BOLA12) or 25.0 g (for 8ASC10) of L-ascorbic acid. After stirring at room temperature for 18 h, the reaction mixture was poured into a 1000 mL beaker containing ice, and stirred until it reached room temperature. The mixture was then transferred to a separatory funnel, and the extraction carried out with ethyl acetate or diethyl ether. The organic phase was washed several times with distilled water and then dried over Na₂SO₄. After evaporation of the solvent under vacuum at 45°C, the product was washed with cold Et₂O or i-octane, in which it shows poor solubility. After filtration, the products were obtained as white solids. Both derivatives were tested with Br₂ in acetone to confirm the presence of the ascorbic acid ring in its active reducing form. For BOLA12: TLC: R_f = 0.45 (CH₃CN/H₂O 9:1); mp

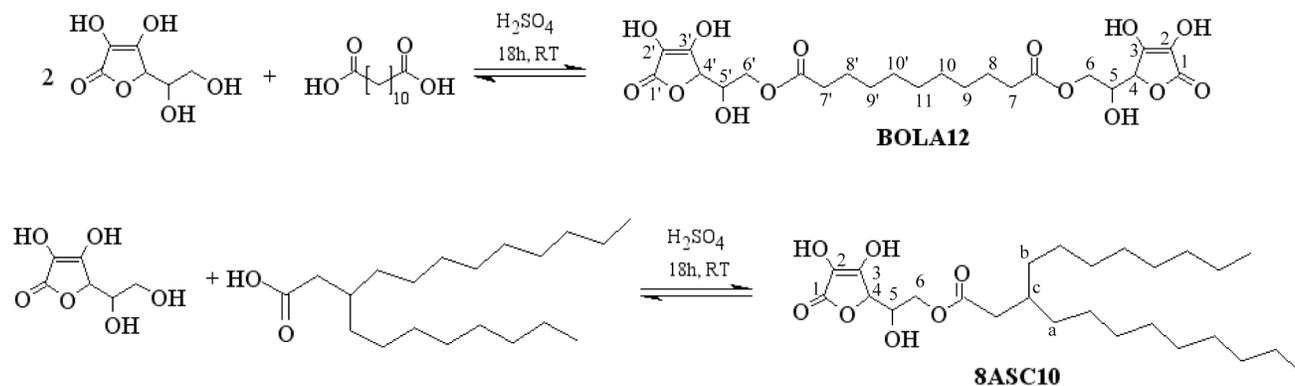


Fig. 1. Synthesis and structure of BOLA12 and 8ASC10.

= 138-140 °C; Elemental analysis: Found C, 52.49; H, 6.90; Calc: (C₂₄H₃₄O₁₄) C, 52.74; H, 6.27; IR (KBr disc) wavelength/cm⁻¹ 3547-3188 (O-H), 2919, 2851 (C-H), 1733, 1691 (C=O), 1646 (C=C), 1470, 1457 (CH₂), 1167 (C-O), 754, 721 (CH₂); NMR δ_H (200 MHz, DMSO; see Fig. 1 for atom numbering) 1.24 (12H, 6×CH₂), 1.39-1.62 (4H, br, 2×H₈, 2×H₈), 2.31 (4H, t, *J* 7.1 Hz, 2×CH₇, 2×H₇), 3.88-4.11 (6H, m, 2×H₆, H₅, 2×H₆, H₅), 4.67 (2H, d, *J*_{4,5} 3.5 Hz, H₄, H₄), 5.30 (2H, br, C₅-OH, C₅-OH), 8.40 (2H, s, C₂-OH, C₂-OH), 11.12 (2H, s, C₃-OH, C₃-OH). For 8ASC10: R_f = 0.30 (CHCl₃/MeOH 95:5); mp = 66.5 °C; Elemental analysis: Found C, 66.64; H, 10.13; Calc: (C₂₆H₄₆O₇) C, 66.35; H, 9.85; IR (KBr disc) wavelength/cm⁻¹ 3586-3100 (O-H), 2927, 2850 (C-H), 1762, 1716 (C=O), 1670 (C=C), 1471, 1340 (CH₂), 1180 (C-O), 743 (CH₂); NMR δ_H (300 MHz, DMSO; see Fig. 1 for atom numbering) 1.26 (m, 7×CH₂), 1.48 (m, CH₂ a and b), 2.35 (CH c), 3.98 (m, 6×CH₂, and 5×CH), 4.66 (d, 4×CH, *J*=1.5 Hz), 5.37 (s, C₅-OH), 8.47 (s, C₃-OH), 11.20 (s, C₂-OH).

2.2 Physico-chemical characterization

Reducing activity. The reducing activity (RA, %) was evaluated by measuring the absorbance at 517 nm of a DPPH (α,α-diphenyl-β-picrylhydrazyl) solution in ethanol (10⁻⁴ mol/L) before (A₀) and after (A₂₀) 20 minutes from the addition of an equal volume of the sample (10⁻⁴ mol/L in ethanol), as RA(%) = 100·(A₀-A₂₀)/A₀.

Differential scanning calorimetry. DSC experiments were carried out with a Q1000 TA Instruments apparatus, using airtight aluminum pans, sealed under nitrogen atmosphere. The transition temperature was taken as the temperature of the peak.

X-ray diffractometry. XRD diffractograms were obtained with a powder Bruker D8 Advance Diffractometer (BRUKER axs), using Bragg-Brentano geometry. λ = 1.5405 Å (CuK_α). Experiments were carried out in the range 1.0° ≤ 2θ ≤ 40°, with a step size of 0.01 degrees, a time/step of 5 sec, setting voltage and current at 30 kV and 20 mA, respectively.

Small angle X-ray scattering. SAXS measurements were carried out with a Hecus SWAX-camera (Kratky) equipped with a position-sensitive detector (OED 50M) containing 1024 channels of width 54 μm. CuK_α radiation (λ = 1.542 Å) was provided by a Seifert ID-3003 X-ray generator (sealed-tube type), operating at a maximum power of 2 kW. A 10 μm-thick nickel filter was used to remove the CuK_β radiation. The sample-to-detector distance was 281 mm. The volume between the sample and the detector was kept under vacuum during the measurements to minimize scattering from the air. The Kratky camera was calibrated using silver behenate. Scattering curves were monitored in a Q-range from 0.014 to 0.5 Å⁻¹. The liquid samples were filled into a 1 mm quartz capillary using a syringe, whereas pastes were filled into a solid samples holder with thin Kapton windows, a 1 mm stainless steel spacer and a Kalrez o-ring (perfluoroelastomer from Dupont). The temperature was controlled with a Peltier element, with an accuracy of ± 0.1 °C. A temperature-scan between 20° and 60 °C was performed by using a temperature-controller (KPR) with the MTC-program (Hecus). All scattering curves were corrected for the empty cell contribution (kapton windows or quartz capillary).

Cryo-Transmission Electron Microscopy (cryo-TEM). The cryo-TEM samples were prepared in a controlled environment vitrification system (CEVS) under controlled temperature and humidity conditions. 5 μL of the sample were placed on a holey carbon Cu-grid which had previously been glow discharge treated. In order to study both the fluid and condensed phases, the temperature in the CEVS was kept either above or below the phase transition temperature, respectively. After blotting excess liquid with a filter paper, the sample was left on the grid for either 10 seconds or a few minutes before plunging. The samples were imaged under cryogenic conditions with a Philips CM120 Biotwin Cryo electron microscope and images recorded with a CCD camera (Gatan 791).

Conductivity. Conductivity measurements were performed using an Ω Metrohm 712 Conductometer with a 0.88 cm⁻¹ cell constant. The transition temperature was determined on a 4% w/w BOLA12/water sample, by measuring its

conductivity as a function of temperature. The critical aggregation concentration was determined by measuring the conductivity of different BOLA12/water samples with a surfactant concentration spanning from $5 \cdot 10^{-2}$ to 5.5% w/w at 48 °C.

Scanning Electron Microscopy. SEM experiments were performed with a Stereoscan S360 (Oxford-Cambridge, UK) equipped with an EDS (Energy Dispersive Spectrometry), INCA X-sight (Oxford instruments, UK), analysis apparatus.

Rheology. Rheology measurements were made with an Anton Paar Physica UDS 200 stress controlled rheometer. A plate-plate geometry was used (20 mm diameter, 0.3 mm gap) with serrated surfaces. The frequency sweep measurements were performed from 0.001 to 15 Hz. The rheological behavior was determined by measuring the G' and G'' moduli in an oscillation regime (the amplitude of the oscillation was 0.5%) in the linear viscoelastic region of deformations. The temperature was 20.00 ± 0.01 °C.

3 RESULTS AND DISCUSSION

3.1 BOLA12

Once dispersed in water at room temperature and above 0.5% w/w, BOLA12 forms opaque condensed phases that – upon heating – turn into transparent homogeneous solutions. For the condensed-to-liquid phase transition, conductivity experiments indicated a value of 33.6 °C for the transition temperature. And a 48 °C the CAC was about 10 mM.

Cryo-TEM micrographs taken on a 5% sample of BOLA12 in the condensed phase show the presence of tubular structures (see Fig. 2). The average external and internal size of the nanotubes is about 280 Å and 150 Å, respectively. The length ranges more or less between 200 nm and 1 µm. The rods are empty, as indicated by the almost electron-transparent central region [5].

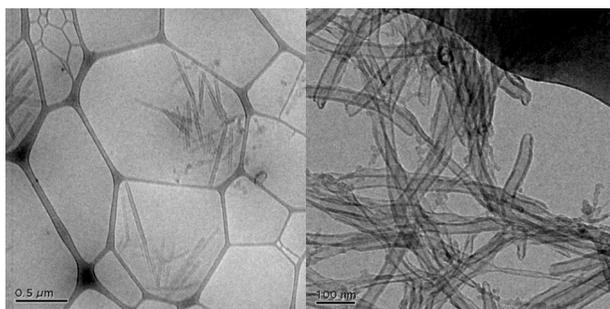


Fig. 2. Cryo-TEM micrographs on a BOLA12, 5% w/w sample in the condensed phase.

XRD experiments (see Fig. 3) show a spacing of 25 Å for the dry powder, that corresponds to the length of the hydrophobic chain in its fully stretched conformation. For all hydrated samples with a water content ranging between 60% and 90%, the spacing shifts up to 40 Å. These values

and the amount of strongly bound water, calculated from DSC experiments [4], support the existence of thin aqueous compartments (about 15 Å in thickness) between adjacent monolayers of the bolaform derivative (see Figure 4).

SAXS data indicate that the condensed hydrated phase contains lamellae with a repeating spacing of 34 Å, and an interlayer of solvating water of about 9 Å. Other peaks appear at 0.022 - 0.025 Å⁻¹, that correspond to a spacing of about 230-250 Å. The nanostructures that produces these peaks comprise hollow cylinders with a wall made up of 2-3 layers of hydrated BOLA12 units, as depicted in Figure 4.

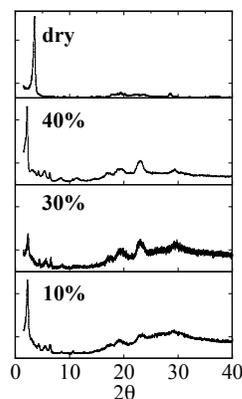


Fig. 3. XRD of BOLA12/water samples.

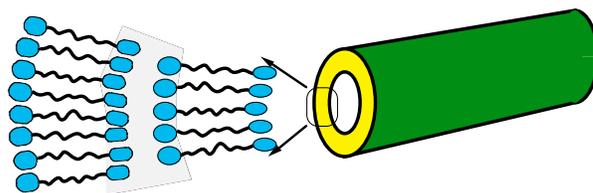


Fig. 4. Multilayer structure of BOLA12 nanotubes. The gray region indicates the interlayer strongly bound water.

The reducing activity of BOLA12 was evaluated according to the DPPH method and was found to be greater than 92%.

3.2 8ASC10

8ASC10 is poorly soluble in water. Instead, in the presence of cyclohexane, it forms birefringent organogels (see Fig. 5).

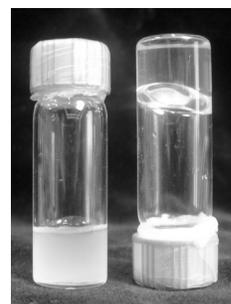


Fig. 5. Organogel from 8ASC10 (5%) in cyclohexane.

The properties of the organogels have been tested for 8ASC10/cyclohexane samples with a gelator concentration ranging between 1 and 5%. The stability of the gel increases with higher amounts of 8ASC10.

The viscoelastic storage modulus (G') and the loss component (G'') of the complex modulus (G^*) of the organogel were measured and plotted as a function of frequency (ω). Their behaviour is typical of a solid-like material: the storage modulus is always much higher than the loss modulus in the observed region of frequencies without a cross-over point of the shear moduli. The results indicate that the physical junctions of the network act as permanent cross-links with long lifetimes, typical of solid systems. The solid-like state is also confirmed by the fact that both G' and G'' are relatively insensitive to the frequency at least in the investigated range (see Fig. 6).

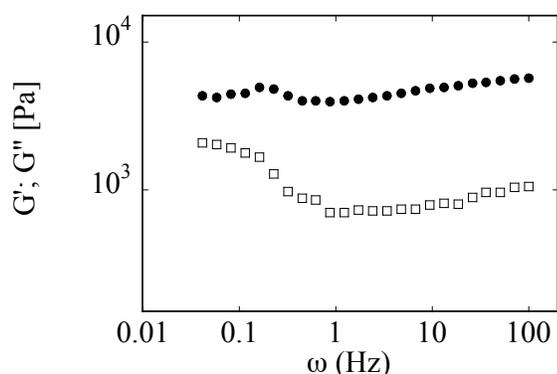


Fig. 6. G' (●), and G'' (□) as a function of ω for a 5% sample of 8ASC10 in cyclohexane.

SAXS experiments (see Fig. 7) indicate the presence of rod-like fibrils with a radius of about 21 Å that corresponds to the length of the surfactant monomer. The 8ASC10 units are arranged in a reversed-micelle order [6], with the hydrophobic tails in the oil phase, and the hydrated headgroups confined in a hydrophilic pool, stabilized by hydrogen bonds.

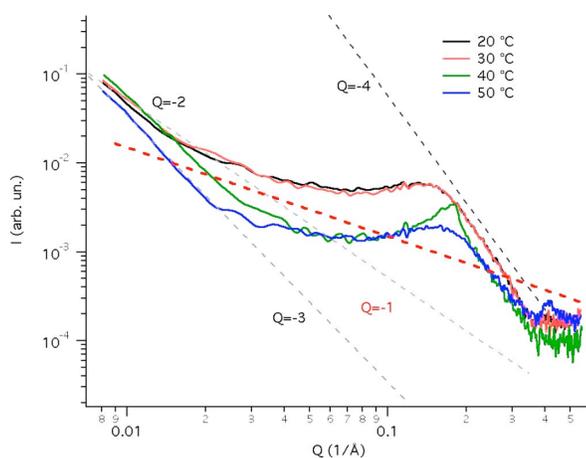


Fig. 7. SAXS profiles of a 8ASC10 (5%) in cyclohexane organogel at different temperatures.

This feature indicates that 8ASC10 in cyclohexane forms “physical gels”, stabilized by attractive van der Waals forces and hydrogen bonding only. The quite low enthalpy change of the sol-gel transition, which occurs at about 35° C, confirms the presence of weak forces.

The reducing activity of 8ASC10 in organogel was evaluated with the DPPH test and was found to be 94%.

4 CONCLUSIONS

We report the synthesis and preliminary characterization of two amphiphilic derivatives of vitamin C.

BOLA12 is a bolaform surfactant that contains two ascorbic acid rings as polar headgroups. This molecule is quite soluble in water, where it forms hollow nanocylinders with large cavities that entrap water.

8ASC10 is a more hydrophobic species, poorly soluble in water, but which forms true organogels in cyclohexane.

Both surfactants and their supramolecular nanoassemblies possess the same reducing activity typical of ascorbic acid. Thus, they can be successfully used as efficient carriers for stabilizing and protecting sensitive hydrophobic molecule, and for increasing their availability in aqueous or oil-based environments.

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