1. Introduction

Previously, we extensively investigated the solid-state and self-assembly properties of amphiphilic single-chained alkanoyl-6-O-ascorbic acid esters (Di-ASC \( n = 8, 10, \text{and} 12 \)) reported: dialkanoyl-5,6-chained amphiphilic compounds obtained from vitamin C are synthesized and investigated for comparison. These products are quite insoluble in water and in polar solvents, although they form homogeneous dispersions in cyclohexane. Upon cooling, these dispersions turn into a gel-like phase. Differential scanning calorimetry, FTIR spectroscopy, and small- and wide-angle X-ray scattering experiments are performed to investigate the properties of pure solids and their liquid dispersions. Di-ASC \( n \) retain the same redox properties of the parent molecule and represent a valid candidate for the production of nanosized protective carriers for valuable guests that are sensitive to oxidative radical attack. Moreover, the contribution of the vitamin C hydroxyl group in position 5 to the overall hydration properties of single- and double-chained amphiphilic derivatives is discussed.

**Organogels from Double-Chained Vitamin C Amphiphilic Derivatives**

Damiano Tanini, [a] Moira Ambrosi, [a] Emiliano Fratini, [a] Antonella Capperucci, [a] and
Pierandrea Lo Nostro \([a, b]\)

The syntheses and physicochemical characterization of double-chained amphiphilic compounds obtained from vitamin C are reported: dialkanoyl-5,6-O-ascorbic acid esters (Di-ASC \( n = 8, 10, \text{and} 12 \)). The acetyl-5-dodecanoyl-6-ascorbic acid ester is synthesized and investigated for comparison. These products are quite insoluble in water and in polar solvents, although they form homogeneous dispersions in cyclohexane. Upon cooling, these dispersions turn into a gel-like phase. Differential scanning calorimetry, FTIR spectroscopy, and small- and wide-angle X-ray scattering experiments are performed to investigate the properties of pure solids and their liquid dispersions. Di-ASC \( n \) retain the same redox properties of the parent molecule and represent a valid candidate for the production of nanosized protective carriers for valuable guests that are sensitive to oxidative radical attack. Moreover, the contribution of the vitamin C hydroxyl group in position 5 to the overall hydration properties of single- and double-chained amphiphilic derivatives is discussed.

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Supporting Information and the ORCID identification number(s) for the author(s) of this article can be found under:
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water to these organic solutions results in the formation of reversed lamellar phases.\[21] We also synthesized similar derivatives from d-isoascorbic acid through the same reaction, and studied the effect of chirality of the polar head group in their phase behavior and self-assembly properties.\[28, 29] Our aim in this work is twofold: 1) we report on the physicochemical characterization of new double-chained surfactants, namely, dialkanoyl-5,6-O-ascorbic acid esters (Di-ASC\(_n\); \(n = 8, 10, \) or 12; see Figure 1), performed through differential scanning calorimetry (DSC), FTIR spectroscopy, and small- and wide-angle X-ray scattering (SAXS/WAXS) measurements; and 2) we discuss the relevance of the hydroxyl group in position 5 of the ascorbic acid ring in determining the hydration properties of these surfactants.

For the latter, we also investigated the properties of 5-acetyl-6-O-dodecanoyl L-ascorbic acid (Ac-ASC12), in which the hydroxyl group in position 5 was replaced by a short acetyl moiety. Our results show that functionalization of this \(\text{-OH}\) group results in a dramatic loss of polarity and hydration of the hydrophilic head, and in a significant reduction of the intermolecular HB capability.

2. Results and Discussion

The packing parameter, \(p\), for a given amphiphile is calculated from Equation (1):

\[
p = \frac{v}{(A_p)}
\]

in which \(v\), \(l\) and \(A_p\) are the volume and length of the hydrocarbon chains and the area per polar head group, respectively. The value of \(p\), which depends only on the geometric features of the amphiphile, can be used to predict the shape of the self-assembled structure produced in a suitable solvent.\[30\] The values of \(v\) and \(l\) are calculated according to the Tanford formulae (Eqs. (2) and (3)):\[31\]

\[
v = 27.4 + 26.9(n - 1)
\]

\[
l = 1.5 + 1.265(n - 1)
\]

in which \(n\) represents the number of carbon atoms in the chain. The \(p\) values for Di-ASC\(_n\) are all close to one; this indicates that these amphiphiles tend to form planar bilayers.

For all Di-ASC\(_n\), \(A_p\) was taken as 45 \(\text{Å}^2\). The previously determined cross-section areas for single-chained L-ASC\(_n\) esters range from 42 to 49 \(\text{Å}^2\), as obtained by surface tensiometry or XRD analysis.\[28\] In the present case, we argue that the presence of a second alkyl tail in position 5 reduces further the polar head group area for two reasons: 1) the conversion of the free \(-\text{OH}\) into an ester group in position 5 reduces polarity and weaker hydration, and a lower intermolecular HB capability, resulting in a smaller \(A_p\); and 2) by comparing L-ASC\(_n\) and their d- counterparts, we observed in previous studies that \(A_p\) was always smaller in the d-isoascorbic mono-alkyl esters than that in the l-ascorbic analogues with the same chain length because the former possessed more intramolecular HB, whereas the latter establish more intermolecular HB.\[28\]

The redox and acidic properties of L-ascorbic acid and its derivatives appear to be related to the furan ring, enediol group, and to the carboxyl in position 1. On the other hand, the biological effects of these molecules are mainly related to the side chain.\[28, 32, 33\] More particularly, the remarkably different biological activity of L-ascorbic and d-isoascorbic acid reflects their different hydration properties and different perturbation they induce in the dynamic structure of neighboring bulk water.\[29, 34–37\]

All Di-ASC\(_n\), in particular, one-armed Ac-ASC12, show negligible solubility in water because the second aliphatic tail increases their hydrophobic character. Additionally, esterification of the secondary hydroxyl group in position 5 removes the hydration capability of the free \(-\text{OH}\) moiety, and thus, alters the set of hydrogen bonds established by the head group with the surrounding environment.

All derivatives are soluble in chloroform and acetone, whereas the solubility in cyclohexane depends on the chain length: Di-ASC8 is completely soluble up to 3 wt.%, Di-ASC10 forms stable turbid suspensions, and Di-ASC12 produces viscous opaque samples.

The samples were investigated through DSC and SAXS to assess their physicochemical properties, thermal behavior, and structural features.

2.1. Characterization of the Pure Solids

2.1.1. DSC

The thermograms recorded for pure Di-ASC8, Di-ASC10, Di-ASC12, and Ac-ASC12 are reported in Figure 2. The temperature \((T_m)\), enthalpy \((\Delta H_m)\), and entropy \((\Delta S_m)\) of melting of the double-chained derivatives are listed in Table S1 in the Supporting Information, together with those of the corresponding alkanes, alkanols, alkanolic acids, ethyl alkanoates, single-chain ascorbyl esters (L and d), and for the bola derivatives (L, O, and O) we reported previously.\[refs.\]

Figure 1. Structures of Di-ASC12 (A) and Ac-ASC12 (B). C) Minimized structure of Di-ASC12 and atom numbering for the ascorbic acid ring. Gray, red, and white spheres represent carbon, oxygen, and hydrogen atoms, respectively.
Upon heating, Di-ASC8 and Di-ASC10 produce two partly overlapped endothermic peaks centered at 64.5 and 73.9 °C for the former and at 45.2 and 47.0 °C for the latter. The two peaks correspond to the melting of two polymorphs (a and b) that coexist in the solids. The same phenomenon is also found in the case of one-armed Ac-ASC12, with two peaks at 73.0 and 100.6 °C. We return to this observation later when we discuss the SAXS and FTIR spectroscopy results.

Instead, in the case of Di-ASC12 only one endothermic melting peak appears at about 46.2 °C.

The behavior of the single-chained surfactants is very different from that of their double-chained counterparts. For the former, the melting temperature and enthalpy change regularly increase with the chain length, as expected. On the other hand, for double-chained Di-ASCn, the melting temperature decreases upon increasing the length of the alkyl chain from 8 to 10 and then it remains approximately constant.

The entropy of fusion shows the highest value for alkanes at constant n; this reflects the high degree of disorder of the liquid state with respect to that in the solid phase. In the case of the alkanols, alkanoic acids, and esters, we recorded more complex behavior related to the effect of different head groups and their capability to establish HB.

Figure 3 shows the variation of ΔS_fus as a function of the alkyl tail length and illustrates that, for the alkanes, alkanols, alkanoic acids, and L- and D-ASC derivatives, the entropy change of melting increases more or less regularly with the number of carbon atoms in the chain.

This is generally ascribed to the effect of the longer alkyl chain and to the different set of HB interactions present in the crystals of L-ASCn, D-ASCn, alkanoic acids, and alkanols. The opposite trend is found for the double-chained derivatives Di-ASC. Such an effect is probably due to weaker interactions that are established between the head groups in the solid state and to the particular arrangement of aliphatic chains in the crystalline structure, as discussed later.

2.1.2. SAXS

SAXS profiles reveal the presence of lamellar phases (Figure 4). For Di-ASC8 (black in Figure 4), the two diffraction peaks are not related to a single lamellar lattice, but show the presence of two coexisting phases with characteristic spacings of 16.6 and 27.3 Å. The SAXS profile for Di-ASC10 (red in Figure 4) shows the presence of a well-defined structure with a dimension of 17.4 Å.

Moreover, we observe a very broad feature at about 0.2 Å⁻¹, presumably related to a second phase, the melting of which is observed in the DSC scans. The three peaks that appear in the case of Di-ASC12 (blue in Figure 4) are in full agreement with a single lamellar phase with a dimension of 35.7 Å. Finally, Ac-ASC12 (green in Figure 4) shows two diffraction peaks that could reflect the presence of a single lamellar phase with a spacing of 30.6 Å. However, both the remarkable intensity and narrowing of the second peak suggest the coexistence of a second lamellar phase with a dimension of 15.7 Å. Table 1 lists the spacings obtained from the SAXS experiments for the investigated specimens.

In the case of the single-chained derivatives, the spacing of the lamellar structure was found to be 31.4, 34.5, and 38.7 Å.
Table 1. Length of the alkyl chain in its fully stretched conformation (l, in Å, see Eq. (3)), spacings (r, in Å), and tilting angles (ψ) for the two crystalline structures 1 and 2 depicted in Figure 5.

<table>
<thead>
<tr>
<th></th>
<th>l [Å]</th>
<th>r₁ [Å]</th>
<th>ψ₁ [°]</th>
<th>r₂ [Å]</th>
<th>ψ₂ [°]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Di-ASC8</td>
<td>10.3</td>
<td>16.6</td>
<td>33</td>
<td>27.3</td>
<td>43</td>
</tr>
<tr>
<td>Di-ASC10</td>
<td>12.9</td>
<td>17.4</td>
<td>32</td>
<td>31.4</td>
<td>43</td>
</tr>
<tr>
<td>Di-ASC12</td>
<td>15.4</td>
<td>-</td>
<td>-</td>
<td>35.7</td>
<td>44</td>
</tr>
<tr>
<td>Ac-ASC12</td>
<td>15.4</td>
<td>15.7</td>
<td>26</td>
<td>30.6</td>
<td>37</td>
</tr>
</tbody>
</table>

for ASC8, ASC10, and ASC12, respectively. A comparison with the spacings found for double-chained Di-ASCn indicates that the presence of the second hydrocarbon chain brings about a significant contraction of the lamellar thickness. Such structural modification is due to stronger interchain van der Waals interactions, which keep the hydrocarbon tails closer and in a more compact arrangement. Furthermore, functionalization of the –OH group in position 5 reduces the hydration capability of the polar head, which presumably leads to an additional lowering of the lamellar thickness. The spacing of one-armed Ac-ASC12 is further reduced.

2.1.3. WAXS, FTIR, and Polymorphs

The spacings obtained from the SAXS data analysis suggest the presence of crystalline structures with different degrees of interdigitation and/or tilting of the molecules.

Figure 5 illustrates the two different forms in which the Di-ASCn molecules are tilted with (1) or without (2) interdigitation of the aliphatic chains. For different spacings, we calculated the two tilting angles (ψ₁ and ψ₂) by using Equation (4):

\[
\tau = (2h + a)\sin \psi
\]

in which \( h \) is the length of the hydrophilic head group (10 Å) and \( a \) is the extent of interdigitation, that is, \( a = 1 \) for fully interdigitated tails and \( a = 2 \) for no interdigitation. The values of the tilting angles determined by Equation (4) and the lamellar thicknesses obtained by SAXS are reported in Table 1.

Polymorphism is a typical feature of fatty acids and consists of the ability of a molecule to take more than one crystalline form, depending on its arrangement within the crystall lattice.

Each polymorphic form is characterized by a distinct short spacing and the chain-length structure produces a repetitive sequence of acyl chains involved in a unit cell lamella along the long-chain axis.

The polymorphic forms can be identified by means of WAXS and FTIR techniques. Figures S1 and S2 in the Supporting Information show the WAXS profiles and FTIR spectra for the investigated samples in the solid state.

Long-chain alkanes and other organic molecules can form different crystalline phases: hexagonal (\( \alpha \)), orthorhombic (\( \beta \)), triclinic (\( \beta’ \)), and monoclinic (\( \beta'' \)); these produce specific bands between 750 and 710 cm\(^{-1} \) in the FTIR spectra.

Table 2 reports the characteristic experimental features that usually indicate the presence of a polymorph.

Table 2. FTIR and WAXS features for the detection of polymorphs.

<table>
<thead>
<tr>
<th>Form</th>
<th>FTIR(^{28,42,43})</th>
<th>WAXS(^{26,42,43})</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \alpha )</td>
<td>720, 717</td>
<td>4.2, 4.2</td>
</tr>
<tr>
<td>( \beta )</td>
<td>719, 727</td>
<td>3.8, 4.2</td>
</tr>
<tr>
<td>( \beta’ )</td>
<td>717</td>
<td>3.6, 3.8, 4.5</td>
</tr>
<tr>
<td>( \beta'' )</td>
<td>717</td>
<td>19.5, 22.5, 23.9</td>
</tr>
</tbody>
</table>

According to our results, Di-ASC8, Di-ASC10, and one-armed Ac-ASC12 show the presence of both \( \beta \) and \( \beta’ \) polymorphs, whereas only the \( \beta'' \) form is found for Di-ASC12.

2.2. Characterization of the Organogels

2.2.1. DSC

Upon heated, the organogel turns into a clear solution; therefore, the phase transition corresponds to collapse of the gel network. Figure S3 in the Supporting Information shows the thermogram for 15 wt% Di-ASC12/cyclohexane organogel, with a temperature peak at 26.7°C and a transition enthalpy change of 26.1 J g\(^{-1} \).

2.2.2. SAXS

SAXS experiments were performed on Di-ASC12/cyclohexane dispersions at different surfactant contents: 0.25, 2, 5, and 15 wt% at 15°C (see Figures 6 and 7). In another set of experiments, the 5 and 15% samples were first heated to 35°C and then cooled back to 15°C, to investigate the reversibility of the phase transition (see Figure S5 in the Supporting Information).

Figure 6 shows the SAXS profile obtained on the 0.25, 2, 5, and 15 wt% specimens at 15°C. Figure S4 in the Supporting Information shows the SAXS profiles for 5 and 15 wt% DIASC12/cyclohexane organogels,
Information (according to the model depicted in the Supporting Information) are reported in Table 3.

Fitting indicates that the gels are made up of building blocks that comprise approximately three stacked discs. In turn, these consist of lamellae with a hydrophilic core of 18 Å and a hydrophobic layer of about 10 Å. These dimensions are compatible with the calculated length of the head group (10 Å) and the length of the hydrophobic tail calculated according to Equation (3) (15.4 Å).

The disc radius is about 7 nm. The structure of the discs agrees with the assembly depicted by John et al. [44].

A comparison between the third and fourth columns and between the last two columns in Table 3 indicates that the gel state is recovered after cooling. The size differences before and after the heating–cooling treatment are within that of the resolution of the equipment. However, the piled up blocks become slightly larger and thinner after cooling with respect to the same structures in the original gels (see the values of \( R \) and \( n \) in Table 3).

The position of the peak due to the presence of nondispersed solid in the dispersion is the same as that previously determined for pure Di-ASC12 (i.e., \( q = 0.18 \text{ Å}^{-1} \)). The Porod exponent (pow) is compatible with a 2D (lamellar) structure of the solid.

The SAXS curves obtained for the dilute sample (0.25 wt%) at 15 ºC, and for higher Di-ASC12 contents at 35 ºC are reported in Figure 7.

Data analysis was performed by adopting a model (see the Supporting Information) that described the scattering objects as spherical particles with a mean radius of about 10 Å. This finding agrees with the presence of reverse micelles with the hydrophilic core made up of polar head groups, whereas the aliphatic tails are extended in the dispersing phase. This arrangement brings about a negligible contrast between the hydrocarbon chains and solvent molecules, so that only the polar head region (micellar core) is responsible for scattering.

The mean radius of the inverse micelles and polydispersity are reported in Table 4.

At a surfactant content of 0.25 wt%, spherical aggregates are formed in solution at low temperature. This sample does not form a gel upon cooling. For more concentrated samples, the low-temperature organogel turns into micellar particles upon heating.

Moreover, we note that the SAXS curve for the most concentrated sample (15%) cannot be fitted by considering only the

---

Table 3. Structural parameters obtained from the SAXS profiles for the investigated organogel samples shown in Figure 6 and Figure S4 in the Supporting Information (see the Supporting Information for definitions).

<table>
<thead>
<tr>
<th>Sample</th>
<th>2%</th>
<th>5%</th>
<th>5% cool</th>
<th>15%</th>
<th>15% cool</th>
</tr>
</thead>
<tbody>
<tr>
<td>( R )</td>
<td>65</td>
<td>66</td>
<td>75</td>
<td>68</td>
<td>76</td>
</tr>
<tr>
<td>( d )</td>
<td>18</td>
<td>16</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>( n )</td>
<td>3.7</td>
<td>3.6</td>
<td>2.5</td>
<td>2.6</td>
<td>2.4</td>
</tr>
<tr>
<td>( q_0 )</td>
<td>0.18</td>
<td>0.19</td>
<td>0.16</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>pow</td>
<td>-2.1</td>
<td>-2.2</td>
<td>-2.2</td>
<td>-2.3</td>
<td>-2.2</td>
</tr>
</tbody>
</table>

---

Figure 6. SAXS profiles for 0.25, 2, 5, and 15 wt% Di-ASC12/cyclohexane dispersions at 15 ºC.

Figure 7. SAXS profiles for 0.25 wt% Di-ASC12 dispersion at 15 ºC (blue), and 2 (green), 5 (red) and 10 wt% dispersions at 35 ºC.
spherical form factor due to the onset of an intermicellar interaction, as shown by the very broad peak at about 0.135 Å⁻¹. This behavior is similar to that previously found for a double-chained mono-substituted ascorbyl derivative (8ASC10). The complete modeling of these curves is out of the scope of this paper.

3. Conclusions

Vitamin C based surfactants with two saturated alkyl chains with 8, 10, or 12 carbon atoms in positions 5 and 6 of the l-ascorbic acid ring (Di-ASCn) were synthesized and investigated. Their water solubility was very poor, although they were able to dissolve and aggregate in organic solvents. Similar to other ascorbyl derivatives, Di-ASC12 formed organogels in cyclohexane that turned into a micellar dispersion upon heating.

The most significant result of this study, however, was the observation that the –OH group in position 5 of ascorbic acid had a key role in imparting water solubility to ascorbyl amphiphiles. In the single-chain ASCn surfactants, the hydroxyl group in position 5 was free and could establish intermolecular hydrogen bonds that determined the stability and thermal of the self-assembled nanostructures. Therefore, the functionalization of the C5–OH group with another fatty acid chain in Di-ASCn not only increased the hydrophobic character of the entire surfactant, but it dramatically reduced its hydration and the possibility of interacting with other molecules through HB. Because the side chain in l-ascorbic and d-isonascorbic acids is usually considered the major incomplete sentence, please check.

Experimental Section

Synthesis and Sample Preparation

All reactions were performed under an inert atmosphere of N₂. All chemicals used were received without further purification. (R)-3,4-Bis(benzoyloxy)-5-{[(5S)-1,2-dihydroxyethyl]furanyl-2(SH)-one was prepared according to a procedure reported in the literature (see the Supporting Information).\[10,\[11] Flash column chromatography purifications were performed with silica gel 60 (230–400 mesh). TLC was performed with TLC plates coated with silica 376 gel 60 F254. NMR spectra were recorded in CDCl₃ on Varian Gemini 200 and Mercury 400 spectrometers operating at 200 and 400 MHz for 1H, and 50 and 100 MHz for 13C.

SAXS and WAXS

SAXS measurements were performed with a HECUS 53-MICRO camera (Kratty-type) equipped with two position-sensitive detectors (OED 50 m) containing 1024 channels of 54 μm in width. Cu Kα radiation (λ = 1.542 Å) was provided by an ultrabright point microfocus X-ray source (GENIX-Fox 3D, Xenoxx, Grenoble), operating at a maximum power of 50 W. The sample-to-detector distance was 281 mm. The volume between the sample and detector was kept under vacuum during the measurements to minimize scattering from the air. The Kratky camera was calibrated in the small-angle region by using silver behenate (5.834 Å), whereas lupolen (4.12 and 3.8 Å) was used as a reference for the wide-angle region. SAXS curves were obtained in the scattering vector (q) range between 0.01 and 0.54 Å⁻¹, in which q = (4π/λ)sinθ/2θ is the scattering angle. The WAXS region covered in the experiment was from 1.3 to 1.9 Å⁻¹. Samples were filled into 1.5 mm glass capillaries. Solid samples were investigated at 25 °C, whereas gel samples were analyzed both below and above the transition temperature. For the latter, the temperature was set to 15 and 35 °C, respectively, and controlled by a Peltier element, with an accuracy of ±0.1 °C. All scattering curves were corrected for the empty-cell contribution by considering the relative transmission factor.

DSC

Calorimetric measurements were performed on a Q1000 differential scanning calorimeter (TA Instruments). Samples were prepared by using hermetic aluminum pans sealed under a nitrogen atmosphere. The phase-transition temperature, T, was taken as the temperature of the endothermic peak, both for solid melting and for gel transition. The enthalpic change, ΔH, was determined by integrating the heat flow curve. All runs were performed at a rate of 2 °C min⁻¹.

FTIR spectroscopy

FTIR experiments were performed by using a Nexus 970-FTIR spectrometer (Thermo-Nicolet) with a KBr beam splitter and MCT/A detector. The instrument was equipped with an attenuated total reflectance (ATR) crystal, with a measuring bridge and a stamp to press the sample on the diamond. The energy range investigated was between 4000 and 650 cm⁻¹. The spectra were recorded with a resolution of 8 cm⁻¹ and coadding 64 scans. All spectra were background corrected.

Reducing Activity

The reducing activity (RA, %) was evaluated by measuring the absorbance at λ = 517 nm of a solution of α,α-diphenylpicrylhydrazyl (DPPH) in ethanol (10⁻⁴ M) before (A₀) and after 20 min (Aₐₐ). The reduction of 20% from the addition of an equal volume of the sample (10⁻⁴ M) in ethanol. RA is defined by Equation (5):

RA(%) = (A₀ – Aₐₐ)/A₀

All derivatives possess a RA value larger than 95%, comparable to those of l-ascorbic acid and its derivatives.[15]
Acknowledgements

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Conflict of interest

The authors declare no conflict of interest.

Keywords: amphiphiles · gels · hydrogen bonds · polymorphism · surfactants


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**ARTICLES**

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**Surfactant stacks:** Lamellar discs are observed in organogels obtained from double-chained vitamin C surfactants. The figure depicts the sequence of hydrophobic (yellow) and hydrophilic (blue) layers, as determined by small-angle X-ray scattering analysis.

**Structures of surfactants derived from vitamin C form organogels @UNI FIRENZE**

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