Solubilization of Hydrophobic Drugs in Octanoyl-6-O-Ascorbic Acid Micellar Dispersions

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ABSTRACT: Alkanoyl-6-O-ascorbic acid esters are easily obtained from vitamin C, and produce self-assembled aggregates in water solutions, with an inner hydrophobic pool surrounded by an external hydrophilic shell. Compared to ascorbic acid, their solubility in oils and fats is greatly enhanced, while the peculiar antioxidant activity is retained in the polar head groups of such surfactants. In virtue of their amphiphilic nature, ascorbic acid-based supramolecular systems can dissolve relevant amounts of hydrophobic, poorly water soluble chemicals such as drugs, vitamins, and so on, and at the same time they provide a suitable shield against oxidative deterioration of valuable materials. In this article we report our study on the self-assembling properties of octanoyl-6-O-ascorbic acid in water, and on the solubilization of some lipophilic molecules in its dispersions. © 2002 Wiley-Liss, Inc. and the American Pharmaceutical Association J Pharm Sci 91:1810–1816, 2002

Keywords: micellar solubilization; vitamin C; ascorbic acid ester(s); micelle(s)

INTRODUCTION

Vitamin C rapidly degrades to even slight heating, light, and action of oxidizing agents.[1] In aqueous solutions it reacts with dissolved oxygen, behaving as an electron donor and producing dehydroascorbic acid (Figure 1), that eventually further degrades.[2] The C_1, C_2, and C_3 carbon atoms are in a tautomeric equilibrium[1] as shown in Figure 1.

Ascorbic acid and its derivatives play an important role in many biological processes as reducing agents or radical scavengers. Triplet excited state carbonyl compounds react with ground state oxygen to give the highly reactive singlet excited molecular oxygen, that reacts with proteins, nucleic acids, and cellular lipids, and induce severe damages in biological systems.[3] When water is exposed to ionizing radiation, different radicals are formed, such as $^{\cdot}OH$ and $^{\cdot}OOH$.[1] The current increment of radical species in the atmosphere (particularly nitrogen oxides), the seasonal partial loss of the ozone layer, and the increase of the ultraviolet irradiation over the Earth, justify the importance of using antioxidant species in protecting terrestrial life from free radicals' attack, or in minimizing their dangerous activity.[4–6]

Cellular protection against singlet oxygen damage may be granted by a number of quenchers, such as ascorbic acid, carotenoids and phenols,[7] notably tocopherols.[8] Ascorbate behaves as a weak singlet oxygen quencher, it is, in fact, a better one-electron reductant than tocopherol ($E^{\circ\prime} = +0.23$ V). Its lipophilic derivative 6-O-ascorbyl-palmitate is a good antioxidant in model systems and is also effective in cellular systems[9] or against Epstein-Barr virus activation.[10] The presence of one or more hydrophobic chains in

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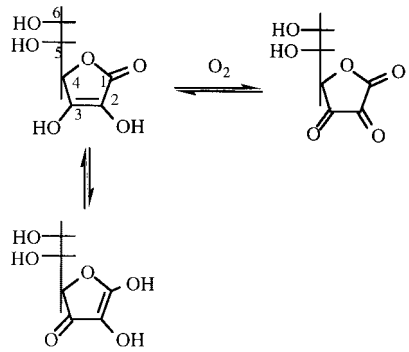

Figure 1. Oxidation and tautomeric equilibrium of ascorbic acid.

vitamin C-based surfactants leads to the formation of supramolecular self-assembled aggregates in water, such as micelles, where an inner lipophilic core is surrounded by a hydrophilic shell made up of the polar headgroups. Several different amphiphilic ascorbic acid derivatives have already been produced as esters, ethers, etc., and their physico-chemical properties reported in the literature.[11–14] Assembled in such supramolecular structures, vitamin C derivatives may protect degradable materials (particularly unsaturated fats or vitamins), by confining the lipophilic solutes in the micellar hydrophobic core, while the ascorbic acid polar headgroups face the water phase and efficiently perform their radical-scavenger activity.

Solubilization of degradable hydrophobic molecules in micellar dispersions or in microemulsions is a valid way for enhancing their concentration and stability in aqueous environments,[15,16] and vitamin C-based surfactants may constitute an interesting class of amphiphilic compounds both for their biocompatibility and for their antiradical activity.

The hydrophobic drugs that we took into consideration in this study are shown in Figure 2. Phenacetin (PHE, *p*-acetophenetidide) is a neutral drug with analgesic and antipyretics pharmacological activities,[17] danthron (DAN,1,8-dihydroxy-anthraquinone) is a stimulant laxative,[17] while anthralin (ANT, 1,8-dihydroxy-anthrone) and retinoic acid (RET) are used against psoriasis.[17] These molecules are poorly soluble in water; ANT and RET need to be stored in airtight containers, protected from light. Anethole (ANE, 1-methoxy-4-(1-propenyl)benzene),carvacrol(CAR, 2-hydroxy-*p*-cymene), thymol (THY, 2-isopropyl-5-methyl-phenol), and eugenol (EUG, 2-methoxy-4-(2-propenyl)phenol) are phenolic molecules

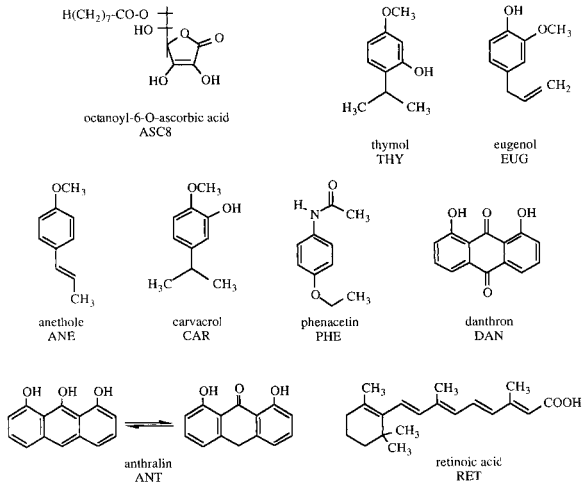

Figure 2. Chemical structure of octanoyl-6-O-ascorbic acid, anethole, carvacrol, thymol, eugenol, phenacetin, danthron, anthralin, and retinoic acid.

usually found in essential oils. They possess a great and wide antibacterial and antioxidant[18,19] activity, but are practically insoluble in aqueous media.[20]

In this article we report a study on the physico-chemical characterization of octanoyl-6-O-ascorbic acid (ASC8) micellar dispersions, and on their capability to dissolve hydrophobic molecules: phenacetin, danthron, anthralin, retinoic acid, thymol, eugenol, anethole, and carvacrol. These hydrophobic, poorly water soluble, unstable compounds were selected to obtain preliminary information on the solubilizing capacity of ASC8 micellar aggregates; the evaluation of the final formulation will be the object of future studies. Antioxidant activity is retained in ASC8 micelles, as DPPH tests and oxygen uptake experiments show.

MATERIALS AND METHODS

Octanoyl-6-O-ascorbic acid (or ascorbyl-octanoate, ASC8) was synthesized according to the procedure already reported in the literature,[13] which involves the reaction between octanoic acid and L-ascorbic acid in concentrated sulphuric acid at room temperature. Purity was assessed through TLC and elemental analysis essays. TLC (silica gel; AcOEt + AcOH): one spot (R_f=0.65). Elemental analyses: C calc. = 55.62%, exp. = 55.38%; H calc. = 7.34%, exp. = 7.30%; m.p. = 87.0–88.0°C. NMR (δ, ppm; DMSO-d^6): 0.85 (triplet, CH_3),

1.23 (CH_2), 1.53 (triplet, CH_2), 2.31 (triplet, CH_2), 4.04–4.06 (multiplet, O–CH_2–CH), 4.67 (singlet, CH), 8.40 (C_2–OH), 11.11 (C_3–OH). UV/VIS (λ_{max}, nm; CH_3CN): 236. IR (KBr, cm^{-1}): 3400, 2918, 2849, 1742, 1690, 1472, 1292, 1179, 1115. The sodium salt was obtained *in situ* by neutralization of a water solution of ASC8 with $NaHCO_3$. All reactants were purchased from Fluka (Milan, Italy), and used without further purification. Bidistilled water was purified with a MilliQ apparatus.

Conductivity measurements were carried out to determine the critical micellar concentration (CMC) and the critical micellar temperature (CMT) with a 712 Metrohn conductimeter, with a cell constant of 0.942 cm^{-1}. The experiments were repeated four times, according to the method reported in the literature.[21,22]

The surface tension (γ, mN/m) of ASC8 aqueous solutions was measured with the Du Noüy ring method at 25° and 30°C. The critical micellar concentration (CMC, mol/L) was determined from the surface tension versus concentration plot at the intersection point of the two straight lines for low and high concentrations, with a thermostatted KSV model Sigma 70 apparatus. The experimental error was ± 1 mN/m. Surface tension experiments were repeated three times and averaged.

Density, refractive index, and viscosity of ASC8 aqueous solutions were measured with an Anton Paar DMA 4500 Densimeter, with an Abbé Refractometer and with an Ubbelhode Viscometer, respectively. All experiments were performed at 30°C. The viscosity of each solution was calculated according to the following formula:

$$\eta = A\rho t - \frac{B\rho}{t}$$

where η is the viscosity of the solution, A and B the instrument constants, ρ is the density of the same sample, and t the outflow time through the viscometer capillary.

The samples for static and dynamic light scattering were filtered through 0.22 μ Millipore filters directly into cylindrical glass tubes. An Ar^+ ion laser source was used, with an incident λ of 514.5 nm. Temperature was controlled with a water circulating bath system, while filtration and circulation of the index-matching liquid (decahydronaphthalene) were performed with a membrane filter and a peristaltic pump. All light scattering experiments have been performed at a scattering angle $\theta = 90°$, and the data analysis was carried out with a Brookhaven Instruments Corporation BI-2030 AT Digital Correlator.

The increment in apparent solubility of some drugs in the presence of micellar solutions of ASC8 was determined through UV-vis spectrophotometry. Aqueous dispersions of ascorbyl-octanoate at different concentrations were saturated with the drug, filtered, diluted (if necessary); the final drug's equilibrium concentration was measured at 250 nm for phenacetin, 427 nm for danthron, and 350 nm for both anthralin and retinoic acid, using a Perkin-Elmer Lambda 35 instrument. The assays were performed in triplicate, and the data are plotted with the sample standard deviation.

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 (A_0) and after (A_{20}) 20 min from the addition of an equal volume of the sample (10^{-4} mol/L in ethanol or water), according to the following formula:[23]

$$RA(\%) = 100 \cdot \frac{A_0 - A_{20}}{A_0}$$

Reducing activity tests were repeated five times on each sample, and the experimental error was $\pm 0.1\%$.

The lipid peroxidation study was performed through UV spectrophotometry on a 0.1 M solution of ethyl linoleate in *n*-hexane;[24] 2.5 mL of the solution were added to the cuvette (pure hexane as blank reference), and a few crystals of AIBN (recrystallized from methanol) were added to start the oxidation of the unsaturated chain. The reaction was monitored by measuring the absorbance at 234 nm, where the diene groups absorb. Finally, 100 μL of an ethanol solution of ASC8 (10^{-4} M) were added to stop the lipid peroxidation. The data were corrected for dilution and the experiment was repeated twice.

The oxygen uptake of ascorbic acid and ASC8 solutions was determined in triplicate, by using an oxygen electrode (mod. DW1, Hansatech, United Kingdom) at 30°C.

RESULTS AND DISCUSSION

The aggregation of ASC8 in water dispersions was studied by measuring the critical micellar concentration (CMC) and the critical micellar temperature (CMT) through conductivity, light scattering, and surface tension measurements.

Figures 3a and 3b report the conductivity values as a function of ASC8 concentration at 30°C, and as a function of temperature at [ASC8] = $5 \cdot 10^{-2}$ mol/L, respectively. The breaking points in the two plots provide a value for CMC of about $(6.1 \pm 0.1) \cdot 10^{-3}$ mol/L, and for CMT of 19.38 ± 0.12°C (standard deviation).

Ionic surfactants' dispersions show a critical temperature (CMT or Krafft Temperature) above which the surfactant solubility increases very rapidly. This is due to the formation of self-assembled structures from the amphiphilic monomers. Above such temperature, the equilibrium system produces a clear and homogeneous dispersion of self-assembled aggregates, while below this temperature the micellar solution forms a semicrystalline mesophase that is generally referred to as "coagel".[25,26]

Figure 4 shows the surface tension of aqueous solutions of the surfactant as a function of [ASC8], at 25° (full circles) and 30°C (white squares), respectively. From the intersection point of the two fitting lines for high and low concentrations we determined a value for CMC of about $4 \cdot 10^{-3}$ mol/L at 25°, and of $6 \cdot 10^{-3}$ mol/L at 30°C, respectively.

Static light scattering experiments can be used to detect the formation of aggregated particles in solutions, based on the refractive index difference between the oil inner micellar core and the external medium. Measurements were performed at $\theta = 90°$, and at 30°C, as a function of ASC8 concentration, and show that the intensity rapidly increases for concentrations higher than $5.8 \cdot 10^{-3}$ mol/L, confirming the CMC value obtained from conductivity and surface tension data. Dynamic light scattering data performed at different angles ($30° < \theta < 150°$) indicate the presence of nearly spherical aggregates with an average hydrodynamic radius of about 26 ± 1 Å.

From the slope of the γ versus log c plots, the polar head group area a_0 can be determined according to the Gibbs equation for adsorption monolayers at the air/water interface:

$$\Gamma = \frac{-1}{2.303 \cdot n \cdot RT} \left(\frac{\partial \gamma}{\partial \log c} \right)_T$$

$$a_0 = \frac{10^{16}}{N_A \cdot \Gamma}$$

where Γ, R, T, and N_A are the surface excess of the surfactant, the universal gas constant, the absolute temperature, and the Avogadro's number, respectively. n is 1 for nonionic surfactants, and 2 for 1:1 ionic amphiphiles. According to our data, we calculated $a_0 = 76$ at 25°, and 88 Å^2 at 30°C, respectively. From previous SANS (small angle neutron scattering) experiments[27] we obtained a value of $a_0 = 58$ Å^2 that agrees with CPK (Corey-Pauling-Koltun) model calculations. This difference may be ascribed to hydration of the polar head groups, penetration of water molecules in the hydrophobic region, micellar size polydispersity, and also to the different parameters and conditions that are used to determine a_0 in the two techniques (surface tension and SANS measurements). SANS data analysis provided a value for the micellar radius of 25.4 Å, that is in good agreement with the value obtained from dynamic light scattering in this study.

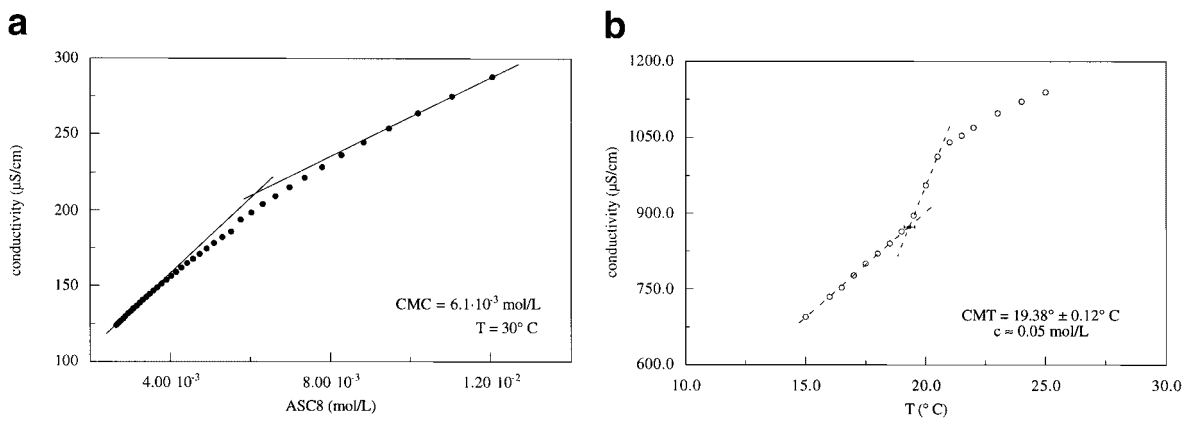

Figure 3. Conductivity measurements as a function of ASC8 concentration at 30°C (a), and as a function of temperature at [ASC8] = 50 mM (b). The plot is the average over four different sets of data; the CMT values have been determined from the intercept of the two straight lines, and the standard deviation (error bar) has been calculated from the four experimental results.

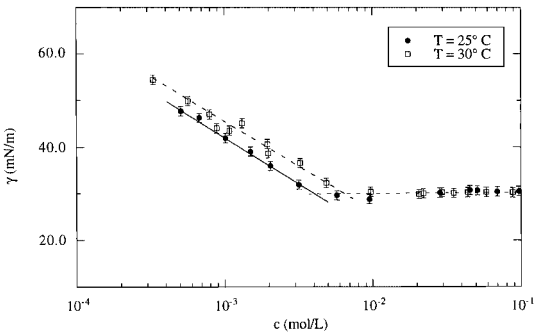

Figure 4. Surface tension of aqueous surfactant's solutions as a function of [ASC8], at 25° (full circles) and 30°C (white squares). Error bars indicate the experimental error for each data.

The apparent solubility of phenacetin, danthron, anthralin, and retinoic acid was measured as a function of [ASC8] and compared to the same value in pure water.

According to Figure 5, a relevant increase of phenacetin apparent solubility is observed (about 22%) with respect to its solubility in pure water ($4.6 \cdot 10^{-5}$ mol/L). The ASC8 concentration corresponding to the initial increment in PHE solubility agrees with the CMC values measured with different techniques. In ASC8 micellar dispersions, DAN almost doubles its solubility ($5.6 \cdot 10^{-5}$ mol/L). Figure 6 shows the solubility increment of anthralin (ANT) and retinoic acid (RET) upon solubilization in ASC8 micellar dispersions. Respect to pure water, their solubility increases of 142 times in the case of ANT, and of 53 times for RET, in the presence of ASC8 micelles. The arrow in Figure 6 indicates the CMC of ASC8 at 30°C, and shows that the solubility of both drugs significantly increases only for concentrations higher than the CMC.

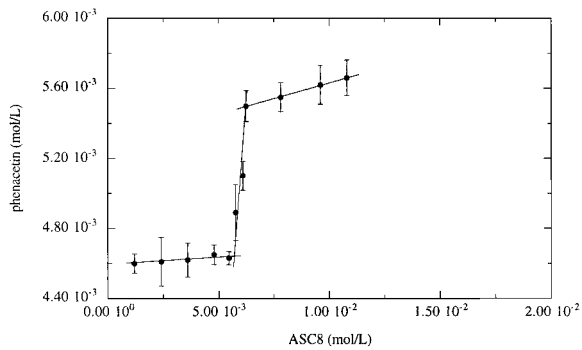

Figure 5. Phenacetin apparent solubility as a function of [ASC8]. Error bars indicate the sample standard deviation.

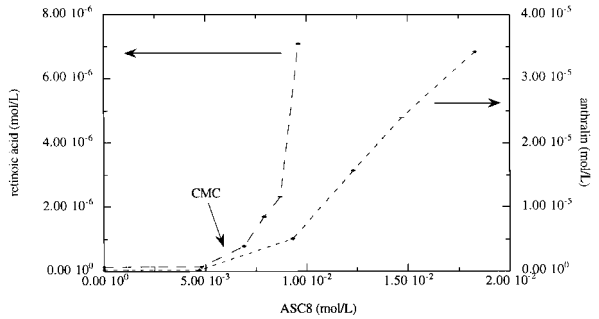

Figure 6. Retinoic acid and anthralin solubility as a function of [ASC8]. For concentrations higher than CMC (indicated by the arrow), the two drugs' solubilities are greatly enhanced. Error bars indicate the sample standard deviation.

Carvacrol, thymol, anethole, and eugenol are practically insoluble in water; when shaken with water, they produce strong turbidity and phase separate in two layers. In the presence of ascorbyl–octanoate micellar solutions (about 7 mM of phenol in 50 mM ASC8) they produced opaque dispersions that did not phase separate even after a year; this finding shows that their dispersions are greatly stabilized by the presence of the amphiphilic ASC8 molecules. Dynamic light-scattering experiments on freshly prepared dispersions indicate the presence of nearly spherical objects with an average hydrodynamic radius of about 40 ± 1 nm.

To check the efficacy of ascorbyl–octanoate aqueous dispersions against oxidative degradation, we carried out some experiments that measure the protective activity performed by ASC8 solutions. Reducing activity was evaluated with the DPPH method, and gave a result of about 89.3% for ASC8 and 90.3% for ascorbic acid. This result shows that ASC8 keeps the same antioxidant properties of vitamin C. Reducing activity measurements were also performed in the emulsified solutions containing carvacrol, thymol, eugenol, and anetol, and provided a value higher than 80% for all chemicals, even after 150 days from the emulsification.

The efficiency of ASC8 in inhibiting lipid peroxidation in hydrophobic environments (where ascorbic acid is not soluble and cannot work as antioxidant) can be easily evaluated by the ability to prevent or inhibit the oxidative degradation of ethyl linoleate in hexane induced by a lipophilic radical initiator, AIBN. As shown in Figure 7, the absorbance at 234 nm increases, due to the formation of conjugated double bonds in the linoleic chain because of the radical attack. The addition

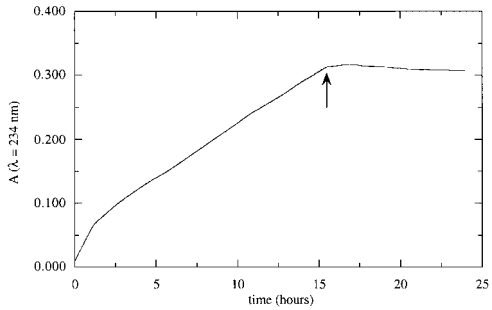

Figure 7. Absorbance at 234 nm after addition of AIBN to a hexane solution of linoleic acid. The arrow indicates the addition of ASC8, that results in the end of the lipid's oxidation.

of an ethanol solution of ASC8 to the sample, indicated by the arrow in the plot, results in the end of the degrading action, and in a constant value of absorbance, even almost 10 h after the addition of the ascorbyl derivative. Finally, oxygen uptake experiments were performed on ASC8 dispersions and compared to ascorbic acid and sodium ascorbate solutions at the same temperature and concentrations. Figure 8 reports the amount of dissolved oxygen, calculated as

$$\log\left(100 \cdot \frac{[O_2]_t}{[O_2]_0}\right),$$

as a function of time t. The linear fitting curves, obtained from triplicates, indicate that ascorbyl–octanoate is less sensitive to oxidation than ascorbic acid and sodium ascorbate. The higher

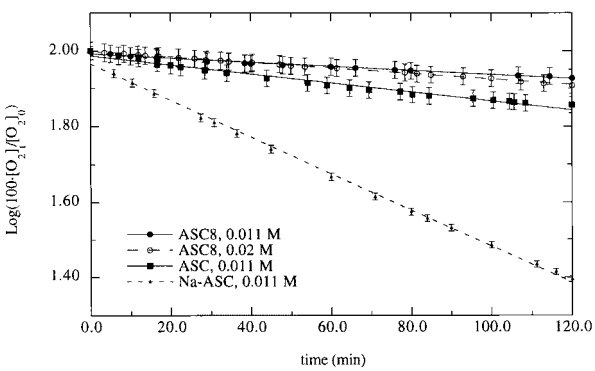

Figure 8. Dissolved oxygen uptake as a function of time for ASC8 (circles), ascorbic acid (squares), and sodium ascorbate (triangles). The curves represent the linear fitting according to the expression: $\log\left(100 \cdot \frac{[O_2]_t}{[O_2]_0}\right) = At + B$, where $[O_2]$ is the concentration of oxygen in the solution at time t. Error bars indicate the sample standard deviation.

reactivity of the salt presumably depends on the fact that alkaline pH values favor the degradation of the ascorbic ring to dehydroascorbic acid.

CONCLUSIONS

Octanoyl-6-O-ascorbic acid (ASC8) combines the amphiphilic nature of a typical short chain surfactant to the antioxidant activity imparted by the ascorbate polar headgroups. In this study we determined the critical micellar concentration and Krafft temperature for ASC8 in water dispersions, by means of conductivity, surface tension, and light scattering experiments. These data are necessary for the characterization of this surfactant's micellar solutions, and for the solubilization of hydrophobic drugs such as phenacetin, danthron, anthralin, and retinoic acid, whose solubilities are greatly increases with respect to pure water. The solubility and stability of emulsified mixtures containing some phenols (anethole, carvacrol, thymol, and eugenol) in water dispersions is also enhanced by the addition of ASC8. Furthermore, the antioxidant activity performed by the ascorbate rings that form the hydrophilic external shells in ASC8 aggregated structures ensures a valid way for protecting degradable materials that have been solubilized in the internal hydrophobic micellar core from oxidative, radical-initiated, attack.

These data can be of interest for increasing the availability of hydrophobic drugs in water-based micellar environments, and with a stronger resistance to oxidation.

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