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Formulation induces Direct DNA UV-A photooxidation. Part II. Pro-oxidant effect of

formulated Vitamin E via generation of singlet oxygen

Johanne Teychené, Dalina Didacus-Prins, Nadia Chouini-Lalanne, Christophe Déjugnat*, Valérie Sartor*

HIGHLIGHTS

- Cetyltriethylammonium bromide (CTEAB) and Vitamin E self-assemble in TRIS/HCI buffer solutions into nanoemulsion composed of CTEAB micelles (that can incorporate Vitamin E) as well as larger Vitamin E droplets.
- CTEAB-Vitamin E nanoemulsion interacts with plasmid DNA pBR322 and the gradual DNA compaction followed by gradual DNA decompaction for increasing CTEAB-Vitamin E amounts have been highlighted.
- UVA irradiation induces photooxidation of DNA on the formulated CTEAB-Vitamin E-DNA lipoplex and underlines the pro-oxidant role of the Vitamin E in this self-assembled system.

1	Formulation induces Direct DNA
2	UV-A photooxidation. Part II. Pro-
3	oxidant effect of formulated Vitamin E
4	via generation of singlet oxygen
5	
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12	
13	Abstract
14	The study focuses on the photosensitization of DNA in nanoemulsion-type systems
15	containing Vitamin E under UVA radiation. Cetyltriethylammonium bromide (CTEAB)
16	surfactant was first combined with Vitamin E to form a CTEAB-Vitamin E nanoemulsion
17	to complex the plasmid pBR322, in TRIS/HCI buffer. The self-organization properties
18	of the CTEAB-Vitamin E system were studied by complementary physico-chemical
19	characterizations (tensiometry, pyrene fluorimetry and scattering experiments). It
20	formed a nanoemulsion composed of CTEAB micelles (that can incorporate Vitamin

21 E) as well as larger Vitamin E droplets. This nanoemulsion was then contacted with 22 DNA and the resulting formulated CTEAB-Vitamin E-DNA lipoplex was irradiated under 23 UVA radiation ($\lambda \ge 335$ nm). The presence of Vitamin E generated a significant 24 enhancement of the DNA photodamage as shown by agarose gel electrophoresis and 25 quantified by photodensitometry. It underlines the pro-oxidant role of the Vitamin E in 26 this self-assembled system. A mechanistic study based on the use of specific 27 scavengers reveals that the singlet oxygen is responsible for the UVA DNA 28 photooxidation in the CTEAB-Vitamin E-DNA lipoplex.

29

30 Keywords

- 31 Surfactant; self-assembly; DNA; UVA photosensitization; Vitamin E
- 32

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1. Introduction

The development of chemical models capable of preventing highly mutagenic lesions induced by UV radiation is a major therapeutic challenge in the field of oncology and more particularly in the field of prevention. The design of alternative systems to protect people from these lesions is therefore a real challenge.

38 Reactive oxygen species (ROS) are permanently generated, transformed, and 39 consumed in all living organisms due to metabolic and biochemical reactions as well 40 as external factors. These species are responsible, directly or indirectly, for many 41 oxidative damage at the molecular level that threaten the stability and integrity of DNA, 42 cell membranes, and proteins. In the cell, antioxidant systems prevent damage caused 43 by free radicals, including alpha-tocopherol, which commonly refers to Vitamin E (VitE) 44 (Fig 1). Thanks to its antioxidant properties against ROS, VitE offers a protective effect 45 to the skin against cell membrane lipid peroxidation and DNA oxidative damage. safeguarding the stability and the integrity of the cell. Therefore, the administration of 46 47 VitE in many pharmaceutical, cosmetic, and food products^{1,2} has been a strategy to decrease DNA damage and ultimately to prevent skin disorders.^{3,4} However, 48 49 prooxidant and antioxidant properties of VitE are still not clearly understood. For 50 instance, Dad and al.⁵ evidenced that alpha-tocopherol is able to generate singlet 51 oxygen, a highly reactive oxygen species responsible in particular for oxidative 52 damage to DNA, when excited in the UVB region at 308 nm. Another work reports the 53 production of endogenous photosensitizer for UVA radiation after exposure of VitE to UVB.⁶ Nocentiny and al.⁷ provided evidence of singlet oxygen production during the 54 55 photolysis of VitE under UVB. These works call into question the VitE functional 56 efficacy as antioxidant.

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58 VitE is a lipid-soluble biological organic compound present in mammalian skin and located in cell membranes (Fig.1). Its use is still limited because of its poor water 59 60 solubility. To overcome this problem, new stable formulations of Vit E have been developed to ensure its encapsulation and transport.^{4,8} Among them, micellar solutions 61 62 are used as delivery systems for active ingredients. Indeed, the solubilization of the 63 active substances into micro- and nanoemulsion-type systems is an efficient way to obtain small sized self-assembled aggregates, allowing the transport of the active 64 65 compound.^{9,10} However, the efficacy of formulated antioxidants depends on the 66 properties of the system: type of emulsion, type of oil, type of surfactant.⁸ These 67 various studies raise the question of the stability of VitE in nanoemulsion-type systems 68 under UVA radiation.

In this context, the purpose of this work is to extend the study of the photosensitization
of singlet oxygen by VitE under UVA radiation in self-assembled systems, and to study
the impact it could have on DNA duplex.

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Fig.1. Molecular structure of α–tocopherol (VitE)

The self-organization properties of cetyltriethylammonium bromide (CTEAB) and the CTEAB-DNA lipoplex in TRIS/HCI buffer have been described in our previous paper.¹¹ Results showed that CTEAB can be used to formulate DNA and pointed out also the effects of compaction on DNA photodamage under UVA radiation which induce the self-photosensibilisation of DNA. 80 In the present study, we developed a cationic nanoemulsion system including 81 CTEAB to deliver the active principle VitE as an integral part of the emulsion. More 82 precisely, we used VitE simultaneously as oil phase and as active ingredient, a kind of 83 2-in-1 self-formulated VitE.

84 In a first part, we present the self-organization properties of CTEAB and VitE in the buffer solution used as solvent for DNA. The determination of the critical 85 aggregation concentration (CAC) (by tensiometry and pyrene fluorimetry) and the 86 87 depiction of the self-assembled aggregates (by scattering experiments) will be shown. 88 Then, the interactions between the CTEAB-VitE formulation - later called as 89 {CTEAB@VitE} – and DNA will be characterized to check that {CTEAB@VitE} can be 90 used to formulate DNA (by tensiometry and pyrene fluorimetry). Finally, the effects of 91 UVA irradiation on these formulations will be investigated by agarose gel 92 electrophoresis. Results will show that the formulations can affect the integrity of DNA 93 due to DNA photooxidative damage. The mechanistic interpretation of the results will 94 be highlighted by the use of peculiar scavengers.

95 **2. Materials and methods**

96 2.1 Materials

97 Cetyltriethylammonium bromide (CTEAB) was synthesized as described in our previous article.¹¹ α-tocopherol (Vitamine E or VitE) in racemic form, heparin sodium 98 99 salt, and sodium azide (NaN₃) were purchased from Alfa Aesar. Agarose and tert-100 butanol (^tBuOH) were obtained from Sigma-Aldrich. Pyrene was purchased from Acros 101 Organics and Ethidium bromide (EB) from Pharmacia Biotech PLUSONE as a 10 102 mg·mL⁻¹ aqueous solution. Eurisotop furnished D₂O and BioLabs the T4 endonuclease 103 V (pyrimidine dimer glycosylase) enzyme. The reagents were used as received. 104 Tris(hydroxymethyl)aminomethane (TRIS) and supercoiled plasmid DNA (Form I) 105 pBR322 (4361 base pairs) were purchased from Thermo Fisher Scientific. The DNA 106 was used after dilution in a 20 mM TRIS/HCl buffer (pH = 7.4) to obtain a stock solution 107 at [DNA-PO₄-] = 4.5×10⁻⁴ mol·L⁻¹. All DNA concentrations indicated in the manuscript 108 are in mol phosphate L⁻¹.

Water used in all experiments was produced by a two-stage Milli-Q filtration
system from Millipore and had a resistivity higher than 18.2 MΩ·cm.

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2.2 {CTEAB-VitE} preparation

112 Colloidal dispersions {CTEAB-VitE} were formulated using a technique based 113 on the preparation of liposomes by the thin film hydration method.^{12,13} Preparations 114 were obtained by mixing CTEAB and VitE, both dissolved in ethanol. The solution was 115 then evaporated to dryness using a rotary evaporator and the film thus obtained was 116 re-dispersed in a 20 mM TRIS/HCI solution (pH = 7.4). The final CTEAB concentration 117 was 8.33×10^{-4} mol·L⁻¹, corresponding to more than 5 times the CMC value of CTEAB 118 in TRIS/HCI (1.5×10^{-4} mol·L⁻¹).¹¹ The final concentration of VitE was 3.58×10^{-4} mol·L⁻¹ 119 corresponding to a VitE / CTEAB concentration ratio of 0.43. This ratio corresponded
120 to the maximal solubility of formulated VitE in the aqueous solution.

121

2.3 Surface tension measurements

Surface tension measurements were performed using a Krüss EasyDyne tensiometer using the Wilhelmy plate method. Temperatures were maintained at 25 °C (± 0.1 °C) by circulating thermostated water through a jacketed vessel containing the solution. For each experiment, surface tension was measured three times (each time ten readings were performed and averaged) after an equilibration time of 30 minutes.

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2.4 Fluorescence measurements

128 A stock solution of pyrene (10⁻⁴ mol·L⁻¹), was prepared in methanol and stored 129 at 4 °C. The solutions for fluorescence probing studies were prepared by adding 1 µL 130 of this pyrene stock solution to 399 µL of the sample solution. Final concentration of 131 pyrene was 2.5×10^{-7} mol·L⁻¹, while the amount of methanol remained very low (0.25 % vol.). Fluorescence measurements were performed using a Fluorolog Horiba 132 133 spectrofluorimeter, with a thermostated cuvette holder at 25 ± 0.1 °C. Excitation 134 wavelength was 335 nm, and the fluorescence emission was recorded from 350 to 500 135 nm.

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2.5 Multi-angle light scattering experiments

137 The measurements were performed on a 3D LS Spectrometer from LS 138 Instruments (Fribourg, Switzerland) equipped with a 100 mW high performance DPPS 139 Laser (Cobolt) operating at $\lambda = 660$ nm, a two-channel multiple tau correlator (auto and 140 cross correlation, 1088 real time channels), a 3D cross-correlation module, a variable-141 angle detection system, two high sensitivity APD detectors, an automated laser 142 attenuation system combined with on-line laser intensity measurement, a temperature 143 controllable sample chamber with index matching vat, and an external circulator for144 temperature control (Julabo CF31).

Before analysis, samples (1.1 mL) were placed into cylindrical scattering cells (10 mm diameter), either directly or after filtration through hydrophilic PTFE filters (450 or 200 nm porosity), as specified in the text.

148 Measurements were performed at 25°C for scattering angles θ ranging from 12° 149 to 150°, corresponding to scattering wave vectors q in the range 2.7×10⁻⁴-2.4×10⁻³ Å⁻ 150 ¹. As samples produced multiple scattering, the 3D cross-correlation configuration was 151 required.¹⁴

At each angle, 3 to 10 measurements were carried out and later averaged after having discarded eventual meaningless correlograms. Analysis of both dynamic- and static light scattering measurements were performed using the home-made MULTI-STORMS software developed by Dr. C. Mingotaud (see *Supporting Information*).

156 **2.6 Small-Angle x-ray Scattering (SAXS) experiments**

157 SAXS measurements were conducted on a Xeus 2.0 SAXS beamline for 158 laboratory from Xenocs (FERMAT federation, Toulouse), with an internal copper 159 source producing 8 keV x-rays (wavelength = 1.54 Å). Signals were recorded using a 160 Pilatus 1M pixel detector from Dectris. The sample-to-detector distance was varied 161 from 387.5 mm to 1687.5 mm so that the covered q range was $0.0042 < q < 0.7 Å^{-1}$. 162 Two scans of 120 min were recorded at each distance and averaged. The observed 163 intensity I(q) was corrected for transmission, solvent, and detector response. Absolute 164 intensity I(q) (cm⁻¹) was obtained after standard normalization. The analysis of the I(q)165 profile was realized using the SasView software (http://www.sasview.org/), version 166 4.2.2.

167 2.7 Irradiation experiments

168 DNA irradiation was made with a Mercury Xenon lamp (Oriel instruments, 169 500W) equipped with a long pass filter $\lambda \ge 335$ nm (Newport 20CGA-335 GC505) at 170 20°C. The irradiated and non-irradiated sample volumes were 100 µL. The final 171 concentrations in TRIS/HCI buffer (20 mM, pH=7.4) were 9×10⁻⁵ mol phosphate·L⁻¹ for 172 the plasmid DNA pBR322, 5×10⁻⁴ mol·L⁻¹ for CTEAB, 2.15×10⁻⁴ mol·L⁻¹ for VitE, 173 200×10⁻³ mol·L⁻¹ for NaN₃, and 4% v/v for ^tBuOH. Each experiment was at least 174 triplicated.

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2.8 Agarose gel electrophoresis

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2.8.1 Sample preparation

177 50 μ L of the sample solution were used in the T4 endonuclease V experiments. 178 It consists to add 10 μ L of T4 endonuclease V (10000 U/ml) to 50 μ L of DNA containing 179 sample and to incubate the solution 30 min at 37°C. 5 μ L of bromophenol blue solution 180 and 10 μ L of Heparin 20% (w/w in TRIS/ HCl buffer (20 mM, pH= 7.4) are then further 181 added.

182 For the samples without T4 endonuclease V treatment, 5 μ L of bromophenol 183 blue solution, 10 μ L of Heparin 20% (w/w in TRIS/ HCI buffer), and 10 μ L of 20 mM 184 TRIS/HCI (pH= 7.4) were added to 50 μ L of formulated DNA.

185

186 **2.8.2 Electrophoresis and photodensitometry**

187 An aliquot of 40 µL of each sample was deposited into 0.8% agarose gel
188 containing 0.02 % of Ethidium bromide. Electrophoresis migration was carried out for
189 4 hours under a constant electric field of 80 V.

Single strand breaks (SSB) were quantified by photodensitometry using the
ImageJ software. To correct the lower efficiency of Ethidium bromide binding to Form
I DNA with respect to Form II, a coefficient of 1.66 was used.¹⁵

193

3. Results and discussion

194 **3.1.** Self-organization of CTEAB and VitE in TRIS/HCI buffer

195 The critical aggregation concentration (CAC) measurement has been carried 196 out by two complementary methods (tensiometry and fluorimetry) in {CTEAB@VitE} 197 mixtures.

Surface tension as a function of CTEAB concentration in presence of Vitamin E
in TRIS/HCI buffer solution is shown in Fig. 2, along with the curve of pure CTEAB for
comparison.



Fig. 2. Surface tension as a function of surfactant concentration in 20 mM TRIS/HCI
 buffer, pH = 7.4, 25°C. Open circles: {CTEAB@VitE} mixture. Dotted line: pure CTEAB.
 ¹¹

205 In the dilute regime, the surface tension value is equal to the one of water and then it 206 decreases as the concentration of surfactant increases to finally reach a plateau. The 207 surface tension curve of {CTEAB@VitE} mixture exhibits two break points. The first 208 one corresponds to the CAC, from which the {CTEAB@VitE} complex is formed. The 209 second one is the CMC of free remaining CTEAB. By this technique, the CAC of 210 {CTEAB@VitE} and the CMC of CTEAB in {CTEAB@VitE} mixture are found to be 211 4.8×10⁻⁵ mol·L⁻¹ and 2×10⁻⁴ mol·L⁻¹ respectively. After the {CTEAB@VitE} complex is 212 formed, at a CTEAB concentration of 4.8×10⁻⁵ mol·L⁻¹, the micellization of free remaining CTEAB in the mixture occurs at a CTEAB concentration of 2×10⁻⁴ mol·L⁻¹. 213 Hence, 4.8×10⁻⁵ mol·L⁻¹ corresponds to the difference between 2×10⁻⁴ mol·L⁻¹ and 214 215 1.5×10⁻⁴ mol·L⁻¹. That means that the CMC of CTEAB in the {CTEAB@VitE} mixture 216 or in pure form remains the same $(1.5 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1})$.

The surface tension in the mixture is lower than that of the pure CTEAB, indicating the formation of a cooperative complex between CTEAB and VitE. The later attracts more surfactant at the air/solution interface.

The aggregation properties studies of {CTEAB@VitE} in TRIS/HCI buffer have also been carried out by pyrene fluorescence measurements. The I_1/I_3 pyrene fluorescence ratio (I_1 at 383 nm and I_3 at 393 nm respectively) are reported in Fig. 3 as a function of CTEAB concentration for {CTEAB@VitE} in TRIS/HCI buffer. For comparison, the I_1/I_3 pyrene fluorescence variation is also represented for CTEAB in buffer solution. To determinate the CAC, results have been adjusted by a sigmoidal Boltzmann function.¹⁶



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Fig. 3. Pyrene I_1/I_3 fluorescence ratio as a function of surfactant concentration in 20 mM TRIS/HCl buffer, pH = 7.4, 25°C (excitation 335 nm). Open circles: {CTEAB@VitE} mixture. Dotted line: CTEAB.¹¹

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The I_1/I_3 ratio in the {CTEAB@VitE} system changes sharply around the CAC, *i.e.* about 2.5×10⁻⁴ mol·L⁻¹. This value is quite close to the 2×10⁻⁴ mol·L⁻¹ value obtained by surface tension measurements. The I_1/I_3 ratio of the pyrene spectra, for high CTEAB concentrations, is directly related to the apparent dielectric constant of the probe environment in the micelles; both values are reported in Table 1.¹⁷

I_1/I_3 ratio (for high CTEAB	Apparent dielectric
concentrations)	constant ϵ
1.5	54.5
1.0	19.2
	I ₁ /I ₃ ratio (for high CTEAB concentrations) 1.5 1.0

238 239

Table 1. Ratio of the first and third vibronic peak of pyrene (I_1/I_3) in micelles as a function of Vitamin E / CTEAB concentration ratio in 20 mM TRIS/HCI buffer, pH = 7.4, 25 °C. Apparent dielectric constant ε calculated from the correlation $I_1/I_3 = 0.0145 \varepsilon + 0.7022.^{17}$

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The dielectric constant value in {CTEAB@VitE} is 19.2, which is much lower than the value obtained in the pure CTEAB solution (54.5). In {CTEAB@VitE}, the probe microenvironment is less polar than in pure CTEAB solution, meaning that the amount of water in the micelles decreases as the VitE concentration increases. Therefore, the addition of VitE promotes the formation of hydrophobic domains in this system.

In summary, close CMC values are obtained whether or not the presence of VitE in solution. This might suggest that VitE has only a slight impact on the selfassembly of CTEAB. However, the presence of VitE creates a less polar environment than in pure surfactant micelles, meaning that water has been ejected from the micelles. Thus, this seems to indicate that the water that hydrated the CTEAB head groups was ejected and replaced by VitE, leading to the formation of mixed CTEAB/VitE micelles.

Further characterizations of the aggregates were conducted by the means ofscattering experiments (light and x-rays).

Multi-angle dynamic- and static light scattering (MADLS and MASLS, respectively) were first performed on the {CTEAB@VitE} system which will be later put in contact with DNA (*i.e.* [CTEAB] = 8.33×10^{-4} mol·L⁻¹ and [VitE]/[CTEAB] = 0.43). The sample was slightly opalescent and preliminary measurements indicated that multiple scattering occurred. For this reason, the 3D cross-correlation technique¹⁴ was used to overcome this problem by discarding the multiple scattering contribution from the total scattered light.

267 In light scattering, the scattering wave vector q is defined as a function of the scattering 268 angle θ as described in (1).

$$q = \frac{4\pi n \sin(\theta/2)}{\lambda} \tag{1}$$

where *n* is the refractive index of the solvent and λ the wavelength of the incident light. MADLS measurements afford the autocorrelation function of the scattered light intensity, normalized over time. A mathematical adjustment then gives access to the decay rate Γ which is related to the translational diffusion coefficient D_0 ($\Gamma = D_0 \cdot q^2$ for a monodisperse system with only translational diffusion). Finally, the hydrodynamic radius R_h is then given by the Stokes-Einstein relationship (2):

$$R_h = \frac{kT}{6\pi\eta D_0} \tag{2}$$

where *k* is the Boltzmann constant, *T* the absolute temperature, and η the solvent viscosity.

The experimental correlation functions (shown in the *Supporting Information, Figure S1*) could be well fitted in the whole range of wave vectors by using a 2nd order Cumulant analysis, which afforded the corresponding decay rates. Plot of the first decay rate Γ as a function of q^2 is presented in Fig. 4A. Obviously $\Gamma = f(q^2)$ was not a linear function, indicating that the relaxation motion recorded by DLS was not solely

282 the translational diffusion coefficient of the scattering particles. It could rather include 283 additional internal motions (such as local rotations for example), probing the particles 284 dynamics at a length scale smaller than their hydrodynamic radius.¹⁸ Even at very low 285 q values, data could only roughly be fitted by a line passing through the origin, whose slope afforded an estimation of an apparent lateral diffusion coefficient $D_{app} \approx 4 \times 10^5$ 286 nm²·s⁻¹, corresponding to an apparent hydrodynamic radius $R_{h,app} \approx 600$ nm. At higher 287 q values, the q^n dependence of Γ (n > 2) could be well described by plotting log Γ = 288 289 $f(\log q)$, showing a linear dependence with slope n = 2.94 (Fig. 4B). This value is very close to the asymptotic Zimm limit (n = 3),¹⁹ which is reached in the case of polymer 290 chains or fractal aggregates under strong hydrodynamic interactions.^{20–22} 291





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Fig. 4. (A): decay rate Γ (s⁻¹) as determined from a 2nd order Cumulant analysis, plotted as a function of q² from MADLS measurements of {CTEAB@VitE}; open circles are the experimental data and the line is the linear fit at very low q values. (B): plot of log Γ as a function of log q showing a linear dependence with a slope very close to 3.

299 As no polymer was present in the {CTEAB@VitE} system, we could hypothesis the 300 presence of a fractal association or a network of strongly (attractive) interacting 301 particles formed by irreversible diffusion-limited aggregation.²³ Therefore, the 302 observed high value of R_h could correspond to long-range assembled structures rather 303 than to elementary aggregates. To get further insights on this self-assembled system, 304 we performed MADLS measurements on samples filtered at 450 nm and 200 nm 305 respectively. Data treatments showed that the slopes in the log Γ = f(log q) curves 306 decreased and got closer to 2. At very low q values, the $\Gamma = f(q^2)$ variation was even 307 linear, allowing more accurate determination of translational diffusion coefficients and 308 hydrodynamic radii, which were found to tend to R_h = 120 nm (Figures S3, S5, 309 Supporting Information). Complementary multi-angle static light scattering (MASLS) 310 characterizations were performed on these samples from 12° to 150° ($q = 2.6 \times 10^{-4}$ -311 2.4×10⁻³ Å⁻¹). Absolute scattered intensities I(q) expressed as Rayleigh ratios (see 312 Supporting Information) are reported in Figure 5.



Fig. 5. Absolute scattered light intensities as obtained by MASLS measurements for
native {CTEAB@VitE} (A) or after filtrations at 450 nm (B) or 200 nm (C). Red dotted
lines are mathematical adjustments.

Regarding the native sample (unfiltered) (Fig. 5A), the scattering profile showed a $q^{-2.3}$ 318 319 regular variation and could be well adjusted using a fractal model. Indeed, the fractal 320 dimension 2.3 determined from the SLS curve corresponds to a compact fractal 321 system.^{24–26} Upon filtration, the scattering profiles changed and exhibited a plateau at low q values followed by a q^{-2} variation at higher q values (Fig. 5B and 5C). This 322 323 suggests that these measurements are better describing individual particles (or 324 primary aggregates) forming the fractal network. General Guinier-Porod fits afforded a radius of gyration R_g = 115 nm, while the scattering profile of the sample filtered at 200 325 326 nm could also be fairly well adjusted using a spherical form factor (R = 143 nm) 327 associated to a sticky hard sphere structure factor (see models descriptions in the 328 Supporting Information). Altogether, these light scattering experiments support the 329 hypothesis of a nanoemulsion system where spherical VitE droplets exhibit strong 330 attractive interactions that lead to a fractal organization at higher scale.

331 Complementary techniques were then used to get further information about this 332 system, namely small-angle x-ray scattering (SAXS). Preliminary experiments 333 indicated that the sample was not concentrated enough to get reliable results. We then 334 had to consider a much more concentrated sample (30 times more concentrated than 335 the initial system). Before SAXS analysis, we first checked by light scattering that the 336 concentrated sample could be representative of the initial one, as not dramatic 337 differences could be evidenced between the two samples by MADLS and MASLS, 338 even upon filtration (Fig. S6, Supporting Information). SAXS measurements are 339 reported in Fig. 6A together with MASLS data recorded on the same sample. The 340 overall spectrum could then be adjusted using a multi-contribution function, including 341 a fractal system and two distinct populations of spherical objects with two different 342 sizes (See *Supporting Information*). The overall description of the system then leads

to a fractal network of large oil VitE droplets together with very small CTEAB empty micelles (R = 1.5 nm) but also swollen CTEAB micelles containing VitE in their core (R=6.5 nm). This multi-scale description is in accordance to what reported in literature in the case of oil-in-water nanoemulsions obtained by a low-energy method such as the solvent inversion technique,^{9,27} and is depicted in Figure 6B.



Fig. 6. (A): Combination of MASLS (blue open circles) and SAXS (black open squares)
measurements of the {CTEAB@VitE} nanoemulsion, and the corresponding overall fit
(red curve) indicating the presence of different aggregates. (B): Schematic
representation of the nanoemulsion formed by the {CTEAB@VitE} system, as deduced
from scattering experiments.

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3.2. Interaction between DNA and {CTEAB@VitE} aggregates

The use of {CTEAB@VitE} aggregates as non-viral vectors for DNA is of concern because of its great potential in gene therapy for the development of protective (antioxidant) formulations. In this context, different techniques were carried out to study the interactions between the {CTEAB@VitE} aggregates and the model plasmid DNA pBR322. The effect of DNA on the {CTEAB@VitE} aggregates was investigated using tensiometry (Fig. 7A) and fluorimetry (Fig. 7B) for increasing CTEAB concentrations (VitE / CTEAB concentration ratio = 0.43).



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Fig. 7. (A): Surface tension measurements of DNA solutions as a function of CTEAB
concentration in {CTEAB@VitE}, in 20 mM TRIS/HCI buffer, pH = 7.4, 25 °C
([VitE]/[CTEAB] = 0.43, [DNA-PO₄-] = 9.10⁻⁵ mol·L⁻¹). Open circles: {CTEAB@VitE} –
DNA mixture. Filled circle: {CTEAB@VitE}–DNA mixture. Cross: DNA+CTEAB
mixture.¹¹

370 (B): Pyrene I_1/I_3 fluorescence ratio in aqueous solution of DNA as a function of CTEAB 371 concentration in {CTEAB@VitE}, in 20 mM TRIS/HCI buffer, pH = 7.4, 25 °C 372 ([VitE]/[CTEAB] = 0.43, [DNA-PO₄-] = 9.10⁻⁵ mol·L⁻¹). Empty circles: {CTEAB@VitE} – 373 DNA mixture. Dotted line: {CTEAB@VitE} mixture.

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The surface tension curve of {CTEAB@VitE}-DNA lipoplex (Fig. 7A) is underneath the two other curves (DNA + CTEAB ¹¹ and {CTEAB@VitE}), suggesting that the complexation between CTEAB, VitE, and DNA has a strong effect on the 378 surface tension. The surface tension of the {CTEAB@VitE}-DNA solution sharply decreases until a first transition appears at a CTEAB concentration of 5.2×10⁻⁵ mol·L⁻ 379 380 ¹ (log C=-4.2), corresponding to the CAC obtained in {CTEAB@VitE} nanoemulsion (4.8×10⁻⁵ mol·L⁻¹). Interestingly, further addition {CTEAB@VitE} between CTEAB 381 382 concentration of 5.2×10^{-5} mol·L⁻¹ (log C=-4.2)) and 3.2×10^{-4} mol·L⁻¹ (log C=-3.5), 383 results in a constant surface tension, suggesting a synergistic aggregation between 384 DNA and {CTEAB@VitE}.²⁸ Then, the surface tension seems to increase slightly and 385 shows a second transition at 3.2×10⁻⁴ mol·L⁻¹ (log C=-3.5), that might correspond to 386 the free DNA depletion from the air/water interface.²⁸

387 To complement these results, pyrene I₁/I₃ emission fluorescence ratio as a function of 388 the CTEAB concentration is presented in Fig. 7B in {CTEAB@VitE}-DNA lipoplex. A 389 roughly sigmoidal shape related to a phase transition and a formation of hydrophobic 390 domains is observed. For low CTEAB concentrations, the I₁/I₃ ratio was found to be 391 1.70 instead of 2.00 in the {CTEAB@VitE} system. This decrease means that pyrene 392 has found to locate in more hydrophobic places. Indeed, different works reported 393 pyrene can interact via partial intercalation with DNA.²⁹ Below the phase transition, 394 the increase in {CTEAB@VitE} concentration is accompanied by an increase in turbidity, observed macroscopically. Previous work¹¹ has shown that the increase in 395 396 turbidity could be associated with strong electrostatic interactions between the DNA 397 and the surfactant, suggesting a gradual compaction of DNA. Progressive compaction 398 refers to a state where individual {CTEAB@vitE} aggregates decorate the DNA and 399 form hydrophobic domains on the DNA strand. The sharp I₁/I₃ ratio decrease can be 400 associated with the critical aggregation concentration that occurs for a CTEAB concentration of 2.5×10⁻⁴ mol·L⁻¹ (log C=-3.6) and which corresponded to the 2nd 401 402 transition observed by tensiometry (Fig. 7A). This CAC evidences again the synergic 403 cooperation between {CTEAB@VitE} and DNA. This remarkable point (Fig.7B, circled 404 point), corresponding to an increase in I_1/I_3 ratio, also matches with a turn from a turbid solution to a limpid solution. This concentration value is in line with the transition 405 observed in surface tension measurements $(3.2 \times 10^{-4} \text{ mol} \cdot \text{L}^{-1})$. The transition from a 406 407 turbid to a limpid solution could indicate the formation of another type of 408 {CTEAB@VitE}-DNA complex due to a large excess of CTEAB. Notably, it could 409 correspond to a DNA decompaction process, as already reported in the literature for 410 excess amount of cationic surfactant.^{30–32} This transition in {CTEAB@VitE}–DNA 411 system appears at much lower CTEAB concentration that the one reported in CTEAB-412 DNA system¹¹ (between 20 and 30 $\times 10^{-4}$ mol·L⁻¹). The presence of vitamin E in the 413 complex seems to promote the DNA decompaction.

The I₁/I₃ ratio value of 0.96, obtained for high {CTEAB@VitE} concentrations in {CTEAB@VitE}-DNA system, corresponds to a micellar dielectric constant of 17, meaning that the pyrene environment is apolar. This dielectric constant value is identical as the one obtained for the {CTEAB@VitE} formulation, suggesting that the presence of DNA does not affect the local micropolarity of pyrene environment, which remains in both systems hydrophobic.

421 3.3. **Photochemistry**

422 Photostability of non-viral vectors is an important concern regarding their topical uses. 423 The {CTEAB@VitE}-DNA lipoplex was formed with a DNA model, the plasmid pBR322, 424 and the nanoemulsion {CTEAB@VitE} described above. Its photostability under UVA 425 irradiation ($\lambda \ge 335$ nm) was studied by agarose gel electrophoresis. Indeed, 426 photodegradation of plasmid pBR322 in supercoiled form (or Form I) induces the 427 formation of single strand breaks (SSB) leading to its relaxed circular form (or Form II). 428 This results in two distinct bands on the agarose gel electrophoresis, noted as I (Form 429 I) and II (Form II) on the gel pictures. In addition, each band can be quantified by 430 photodensitometry.

431 The solutions were irradiated one minute. With this time of irradiation, and in presence 432 of {CTEAB@VitE}-DNA complex, DNA photodamage are well quantified while no 433 overbreaks are observed. (Fig. 8A, lane 6*) UVA irradiation of the {CTEAB@VitE}-DNA 434 lipoplex (Fig. 8A, lane 6*) induces around a threefold increase of the SSB ratio (Fig. 435 8B) compared to CTEAB-DNA complex (Fig. 8A, lane 5*). The presence of Vitamin E 436 on the lipoplex generates a significant enhancement of the DNA photodamage. As the 437 formation of singular oxygen by CTEAB-DNA complex by a Triplet-Triplet energy 438 transfer mechanism has been previously reported,¹¹ we have investigated the 439 formation of damage to DNA that may be caused by such a mechanism, namely the 440 formation of CPD and the formation of singular oxygen to determine the mechanism 441 involved in this photodegradation. First, the samples were treated by a phage T4 442 endonuclease V enzyme (T4), a DNA enzyme repair that cleaves double-strand DNA 443 at cyclobutane pyrimidine dimer (CPD), leading to single strand breaks by a two-step 444 mechanism. In this study, if CPD is formed by photosensitization and the plasmid 445 pBR322 treated with T4, single strand break will be made and the Form II of the

446 plasmid will appear on the electrophorese agarose gel. T4 treatment on non-irradiated 447 plasmid pBR322 reveals the presence of abasic lesions by AP-lyase activity of the T4 448 (Fig. 8A, lane 7 and Fig. 8B). Without irradiation, in presence of CTEAB or 449 {CTEAB@VitE} and with T4 treatment, the SSB ratio decreases dramatically (lanes 8 450 and 9 respectively), as already shown for CTEAB-DNA lipoplex.¹¹ The hypothesis was 451 an enzyme quenching in presence of the CTEAB or the non-access to the DNA double 452 strand to the enzyme because of its compaction. It was also previously observed that 453 after 10 min irradiation, the T4 treatment revealed again the DNA photodamage.¹¹ In 454 these experiments and for a lower time of irradiation (1 min), the photooxidative 455 damage of the plasmid induced by the CTEAB alone are limited (lane 5*). That's what 456 the SSB ratio observed should be due, only to abasic lesions already present in native DNA, and to the CPD formed under irradiation. After T4 treatment of the sample (lane 457 458 11*), the SSB ratio becomes again equivalent to the SSB ratio for DNA alone (lane 459 10*) and the native DNA abasic site level is recovered and no increase of the SSB ratio 460 is observed in presence of CTEAB and one minute of irradiation, compared to DNA 461 alone (Fig. 8B). No CPD are formed and the T4 enzyme works again if the sample is 462 irradiated. The small amount of damage formed by the irradiation seems enough to 463 induce some modification on the DNA compaction and to allow recovering the 464 enzymatic activity of the T4 endonuclease V.



465

466 Fig. 8. DNA cleavage for 1 min of irradiation at $\lambda \ge 335$ nm and 20°C with CTEAB (5×10⁻ 467 ⁴ mol·L⁻¹) or {CTEAB@VitE} after or not phage T4 endonuclease V treatment (T4) (* 468 indicates the irradiated samples). A: Electrophoresis agarose gel of plasmid pBR322 469 $(9 \times 10^{-5} \text{ mol} \cdot L^{-1})$ in 20 mM TRIS/HCI buffer (pH = 7.4). Lanes 1 and 4*: DNA alone; 470 lanes 2 and 5*: DNA + CTEAB; lanes 3 and 6*: DNA + {CTEAB@VitE}; lanes 7 and 471 10*: DNA alone + T4; lanes 8 and 11*: DNA +CTEAB + T4; lanes 9 and 12*: DNA + 472 {CTEAB@VitE} + T4. B: Single strand break (SSB) ratio of DNA, CTEAB-DNA and {CTEAB@VitE}-DNA lipoplexes ± T4 with and without irradiation. 473

474

The same behavior is observed for the {CTEAB@VitE}-DNA lipoplex. Without irradiation and after T4 treatment, no CPD are revealed (lane 9) and with irradiation and T4 treatment, the SSB ratio corresponds to addition of native abasic site level and photooxydative damage due to the irradiation (lane 12*). Thus, no significant CPD
formation is shown in our experimental conditions in presence of the {CTEAB@VitE}
nanoemulsion and under UVA irradiation.

481 Three specific scavengers were also used to understand the mechanism of the DNA 482 photodamage by the {CTEAB@VitE} nanoemulsion. The sodium azide (NaN₃) reveals 483 the role of the singlet oxygen in the DNA photodamage by quenching it, D₂O also by 484 increasing the singlet oxygen lifetime and the *tert*-Butanol (^tBuOH) shows the 485 implication of the hydroxyl radical species by quenching them (Fig. 9). In a previous 486 paper,¹¹ it was shown that singlet oxygen was responsible for the oxidative damage in 487 CTEAB-DNA complex. For the {CTEAB@VitE}-DNA lipoplex under UVA irradiation 488 and with NaN₃ (lane 11^{*}), the SSB ratio observed decreases dramatically, compared 489 to {CTEAB@VitE}-DNA lipoplex alone (lane 10*) and until to be equal to the one for 490 DNA alone (lane 8^{*}). It means that the singlet oxygen is again involved on the 491 photooxydation of the plasmid DNA by the {CTEAB@VitE} nanoemulsion. D₂O was 492 also used to bear out the formation of singlet oxygen and its involvement on the 493 photodegradation process. On lane 13* and Fig. 9B, a slight increase of the SSB ratio 494 is observed for the sample on D₂O. By means of *tert*-Butanol, the involvement of 495 hydroxyl radical species can be shown. In presence of *tert*-Butanol, the SSB ratio stays 496 equivalent to the SSB ratio for the {CTEAB@VitE}-DNA lipoplex alone. The hydroxyl 497 radical species are not involved on the oxidative damage of the plasmid DNA.

498 Consequently, the singlet oxygen is the major responsible for the direct UVA 499 photooxydation of DNA on the {CTEAB@VitE}-DNA lipoplex. The mechanism of this 500 process is a type II mechanism by triplet triplet energy transfer from the 501 {CTEAB@VitE}-DNA lipoplex to the ground state of the molecular oxygen leading to 502 the formation of the reactive singlet oxygen.

503 This study hightlights the role of the vitamin E as a pro-oxidant species on the 504 {CTEAB@VitE}-DNA lipoplex by favoring the direct UVA photooxydation of the DNA 505 duplex.



506

507 **Fig. 9.** DNA cleavage for 1 min of irradiation at $\lambda \ge 335$ nm and 20°C with 508 {CTEAB@VitE} and NaN₃ (200×10⁻³ mol·L⁻¹), ^tBuOH (4% v/v), in D₂O (80% v/v) or 509 phage T4 endonuclease V treatment (T4) (* indicates the irradiated samples). A: 510 Electrophoresis agarose gel of plasmid pBR322 (9×10⁻⁵ mol·L⁻¹) in 20 mM TRIS/HCI 511 buffer (pH = 7.4). Lanes 1 and 8*: DNA alone; lanes 2 and 9*: DNA + T4; lanes 3 and 512 10*: DNA + {CTEAB@VitE}; lanes 4 and 11*: DNA + {CTEAB@VitE} + NaN₃; lanes 5 513 and 12*: DNA + {CTEAB@VitE} + ^tBuOH; lanes 6 and 13*: DNA + {CTEAB@VitE} in 514 D_2O ; lanes 7 and 14*: DNA + {CTEAB@VitE} + T4. B: Single strand break (SSB) ratio

515 of + {CTEAB@VitE}-DNA lipoplex \pm NaN₃, ^tBuOH, D₂O or T4 with and without 516 irradiation.

517 **4. Conclusion**

518 Various studies raise the question of the stability of Vitamin E in nanoemulsion-type 519 systems under UVA radiation. In this work, the photosensitization of singlet oxygen by 520 Vitamin E into self-assembled systems under UVA was studied. The cationic surfactant 521 CTEAB has shown to be an efficient complexing agent to DNA. CTEAB surfactant was 522 then used to form a Vitamin E-containing nanoemulsion that could interact with DNA. 523 The self-organization properties of the CTEAB and vitamin E were studied by different 524 physico-chemical characterizations (tensiometry, pyrene fluorimetry, and scattering 525 experiments). The overall description of the system leads a complex fluid composed 526 of different aggregates ranging from CTEAB micelles to a fractal network of large oil 527 Vitamin E droplets. The study of the interactions between CTEAB-Vitamin E and 528 plasmid DNA highlighted the gradual DNA compaction followed by gradual DNA 529 decompaction for increasing CTEAB-Vitamin E amounts. Vitamin E seems to promote 530 DNA decompaction.

531 Formulated CTEAB-vitaminE-DNA lipoplex were irradiated under UVA light. Presence 532 of vitamin E generates a significant enhancement of the DNA photodamage. The 533 mechanistic interpretation of the results has been highlighted by the presence of 534 peculiar scavengers. It reveals that the singlet oxygen is responsible of the direct UVA 535 photooxydation of DNA on the CTEAB-Vitamin E-DNA lipoplex. This study underlines 536 the role of vitamin E as a pro-oxydant species by favoring the UVA DNA 537 photooxydation. From a safety point of view, a warning is necessary regarding the 538 topical pharmaceutical or cosmetic applications of such a formulation.

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Declaration of interests

I The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

□ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Supporting information for:

Formulation induces Direct DNA UV-A photooxidation. Part II. Pro-oxidant effect of formulated Vitamin E *via* generation of singlet oxygen

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1.	Multi-angle dynamic light scattering (MADLS)	2
2.	Multi-angle static light scattering (MASLS)	6
3.	Light scattering of concentrated samples	8
4.	Analysis of small angle X-ray scattering data (SAXS)	9
5.	References	9

1. Multi-angle dynamic light scattering (MADLS)

In MADLS the measured autocorrelation function $g^{(2)}(q,\tau)$ of the scattered intensity I(q,t) normalized over time is expressed as (1):

$$g^{(2)}(q,\tau) = \frac{\langle I(q,t)I(q,t+\tau)\rangle}{\langle I(q,t)\rangle^2}$$
(1)

It affords the correlation function of the electric field $g^{(1)}(q,\tau)$ using the Siegert relationship (2):

$$g^{(2)}(q,\tau) = 1 + \beta \left| g^{(1)}(q,\tau) \right|^2 \quad \beta \le 1$$
⁽²⁾

In the ideal case of monodisperse spherical particles under Brownian motion, the autocorrelation function decays exponentially over time (3):

$$g^{(1)}(q,\tau) = e^{-\Gamma\tau}$$
(3)

where $\Gamma = D_0 q^2$ is the decay rate and D_0 is the translational diffusion coefficient of the particles. Real situations are more complex and different algorithms can afford the decay rate from $g^{(1)}(q,\tau)$, among them Cumulant, non-negatively constrained least squares (NNLS), and CONTIN analysis. For this purpose, we used a home-made software (namely MULTI-STORMS, written and compiled with MATLAB® by Dr. C. Mingotaud). It offers the choice in the methods used to analyze the correlograms mathematical adjustments are performed using different algorithms based on the shape and the scattering properties of the particles to be analyzed.

The hydrodynamic radius of equivalent sphere (R_h) is then obtained by the Stokes-Einstein relationship (4):

$$R_h = \frac{kT}{6\pi\eta D_0} \tag{4}$$

where *k* is the Boltzmann constant, *T* the absolute temperature, and η the solvent viscosity.

Experimentally, multiple scattering was observed when measuring the {CTEAB@VitE} system (slightly opalescent) and its dilutions. The 3D cross-correlation configuration was then used to eliminate the multiple scattering contribution.¹ The

experimental autocorrelation functions measured on {CTEAB@VitE} are shown in Figure S1. The curves were well fitted with a 2nd order Cumulant analysis² (5):

$$g^{2}(\tau) = B + \beta \cdot e^{-2\overline{\Gamma}\tau} \cdot (1 + \frac{\mu_{2}}{2!}\tau^{2} - \frac{\mu_{3}}{3!}\tau^{3} + \cdots)^{2}$$
(5)

where *B* is the background value, β the amplitude of the autocorrelation function, $\overline{\Gamma}$ the mean value of the decay rates and μ_n the nth moment of the distribution function of decay rates, $f(\Gamma)$. This analysis provided the intensity-weighted decay rates Γ , that did not vary linearly with respect to q^2 . It indicated several contributions in the overall apparent diffusion coefficient D_{app} (translations, internal motions such as rotations, etc.).



Figure S1. Auto-correlation functions obtained from MADLS measurements of the {CTEAB@VitE} system (black curves) and the corresponding fits using a second-order Cumulant analysis (red curves).

Similar measurements were performed in the same conditions on {CTEAB@VitE} samples after filtrations at 450 nm and 200 nm. Correlograms were also well adjusted with a 2nd order Cumulant analysis. Measurements and analysis are shown in Figures S2 and S3 (filtration at 450 nm) and in Figures S4 and S5 (filtration at 200 nm).



Figure S2. Auto-correlation functions obtained from MADLS measurements of {CTEAB@VitE} filtered at 450 nm (black curves) and the corresponding fits using a second-order Cumulant analysis (red curves).



{CTEAB@VitE} filtered @ 450 nm

Figure S3. (left): decay rate Γ (s⁻¹) as determined from a 2nd order Cumulant analysis, plotted as a function of q² from MADLS measurements of {CTEAB@VitE} filtered at 450 nm; open symbols are the experimental data and the line is the linear fit at very low q values. (right): plot of log Γ as a function of log q showing a linear dependence.



Figure S4. Auto-correlation functions obtained from MADLS measurements of {CTEAB@VitE} filtered at 200 nm (black curves) and the corresponding fits using a second-order Cumulant analysis (red curves).



{CTEAB@VitE} filtered @ 200 nm

Figure S5. (left): decay rate Γ (s⁻¹) as determined from a 2nd order Cumulant analysis, plotted as a function of q² from MADLS measurements of {CTEAB@VitE} filtered at 200 nm; open symbols are the experimental data and the line is the linear fit at very low q values. (right): plot of log Γ as a function of log q showing a linear dependence.

2. Multi-angle static light scattering (MASLS)

In MASLS experiments, the excess of scattered intensity with respect to the solvent is converted into absolute intensity (Rayleigh ratio R_{θ} , in cm⁻¹) using toluene as a reference, for which the excess Rayleigh ratio is known ($R_{\theta,toluene} = 1.19 \times 10^{-5}$ cm⁻¹ at 660 nm)³ as described in equation (6):

$$R_{\theta,sample} = \frac{I_{sample} - I_{solvent}}{I_{toluene}} \cdot \left(\frac{n_{solvent}}{n_{toluene}}\right)^2 \cdot R_{\theta,toluene}$$
(6)

where I_{sample} , $I_{solvent}$, and $I_{toluene}$ are the scattering intensities of the sample solution, the solvent, and the toluene respectively, and where $n_{solvent}$ and $n_{toluene}$ are the refractive indexes of the solvent and the toluene at 660 nm, respectively ($n_{water} = 1.33$ and $n_{toluene} = 1.49$ at 25 °C).

MASLS profiles were then fitted using the SasView software (version 4.2.2.) applying different models:

(i) a general, empirical Guinier-Porod fit to estimate the size and the dimensionality of scattering aggregates and defined as (7-9):⁴

$$I(q) = \begin{cases} \frac{G}{q^s} \cdot exp\left\{\frac{-q^2 R_g^2}{3-s}\right\} & q \le q_1 \\ \frac{D}{q^d} & q \ge q_1 \end{cases}$$
(7)

$$q_1 = \frac{1}{R_g} \cdot \left[\frac{(d-s)(3-s)}{2}\right]^{1/2}$$
(8)

$$D = G \cdot exp\left\{\frac{-q_1^2 R_g^2}{3-s}\right\} \cdot q_1^{(d-s)}$$
⁽⁹⁾

where *G* and *D* are the Guinier and Porod scale factors respectively, R_g the radius of gyration, *d* the Porod exponent, and *s* a parameter depending on the geometry (*s*=0 for spheres, *s*=1 for rods, *s*=2 for lamellae or platelets).

(ii) A mass fractal model using the form factor $P(q)_{sphere}$ of a sphere, and described in equations (10-12):⁵

$$I(q) = scale \cdot P(q) \cdot S(q) + background$$
(10)

$$P(q)_{sphere} = 9 \cdot \left[\frac{\sin(qR) - qR\cos(qR)}{(qR)^3}\right]^2 \tag{11}$$

$$S(q) = \frac{\Gamma \cdot (D_m - 1) \cdot \zeta^{D_m - 1} \cdot \sin[(D_m - 1) \tan^{-1}(q\zeta)]}{q \cdot [1 + (q\zeta)^2]^{(D_m - 1)/2}}$$
(12)

where *R* is the radius of the primary particle, D_m the mass fractal dimension, and ζ the cut-off length.

(iii) The sticky hard sphere adjustment was realized combining the form factor of a sphere $P(q)_{sphere}$ (11) and a interparticle structure factor $S(q)_{stickyHS}$ for a hard sphere fluid with a narrow attractive well and using a perturbative solution of the Percus-Yevick closure. The full description of $S(q)_{stickyHS}$ can be found in the literature.^{6,7}

3. Light scattering of concentrated samples

30x {CTEAB@VitE} unfiltered



Figure S6. (left): decay rate Γ (s⁻¹) as determined from a 2nd order Cumulant analysis, plotted vs q² from MADLS measurements. (center): plot of log Γ as a function of log q showing a linear dependence. (right): absolute scattered light intensities as obtained by MASLS. From top to bottom: 30x {CTEAB@VitE} unfiltered, filtered at 450 nm, filtered at 200 nm.

4. Analysis of small angle X-ray scattering data (SAXS)

The general expression of the scattered intensity I(q) is given by equation (13):

$$I(q) = \Phi \cdot V \cdot (\Delta \rho)^2 \cdot P(q) \cdot S(q)$$
(13)

where $q = [4\pi \sin(\theta/2)]/\lambda$ is the scattering vector (θ is the scattering angle), Φ is the volume fraction of the scatterers, V is their volume and $\Delta \rho$ is their difference in scattering length density (SLD) with respect to the solvent. P(q) is the scatterers' form factor and S(q) is the structure factor accounting for inter-particle interactions, for which various analytical models are available.

Combining SLS and SAXS curves, the overall scattered intensity could be adjusted by a combination (14) of a fractal model and two additional sticky hard sphere models (as described above in the MASLS section):

$$I(q)_{total} = I(q)_{fractal} + I(q)_{stickyHS,1} + I(q)_{stickyHS,2}$$
(14)

5. References

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