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# Formulation induces direct DNA UVA photooxidation. Part I. Role of the formulating cationic surfactant

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

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22 especially in the compacted state. A mechanistic study using selective scavengers  
23 shows the involvement of singlet oxygen in these oxidative processes due to the  
24 direct UVA absorption of DNA itself.

25

## 26 **Keywords**

27 surfactant; self-assembly; DNA; formulation; UVA photosensitization

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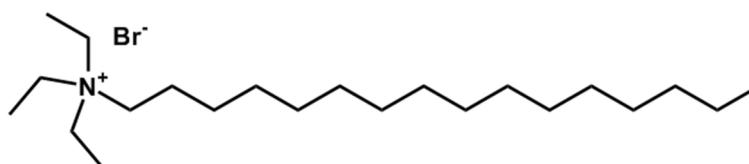
29 *\*corresponding author: Dr. Christophe Déjugnat, dejugnat@chimie.ups-tlse.fr*

## 1. Introduction

If gene therapy opens up new therapeutic perspectives in the treatment of inherited deficiency diseases (*e. g.* cystic fibrosis), acquired diseases (cancer, neurodegenerative diseases) or infectious diseases (hepatitis, AIDS), its success depends on the effective transmission and expression of genetic material within cells. This undoubtedly requires the development of systems capable of efficiently and safely crossing biological barriers on the way to the nucleus and transmitting the drug gene there. Most of the systems used involve recombinant viral vectors, capable of inserting efficiently the transgene into the cells. Although the latter are very effective in the transfer function, their use has a number of disadvantages (production cost, safety, development of immune reactions, and risk of pathogenicity). To avoid them, a number of synthetic vectors have been developed. They are mostly cationic derivatives of a lipidic or polymeric nature capable of interacting with DNA by electrostatic interactions and compacting it to form particles (aggregates).<sup>1</sup> Moreover, since the effective charge of the DNA and its size are reduced, the efficiency of the transfer of the drug gene to its site of action is also improved.

In the case of the *in vivo* delivery, the compacted gene/vector system can be transferred to the target DNA in two ways: either by the systemic route or by local application.<sup>2</sup> However, whatever the mode of administration, the gene must be associated with a system capable of protecting it against aggression by the external environment and in particular from UV radiation, one of the main sources of DNA damage at the origin of photocarcinogenesis processes. While the photomutagenic potential of UVB radiation has long been established, that of UVA radiation, which is longer and deeper penetrating the skin, has only recently been recognized. Indeed, the direct formation of cyclobutane pyrimidine dimers (CPD) in DNA under UVA

55 radiation has been widely reported<sup>3-12</sup> and the involvement of UVA radiation in  
56 cancer and skin aging processes is now well established. However, no study has  
57 been carried out on the direct influence of UVA radiation on DNA in a synthetic vector  
58 system. Yet DNA delivery in the skin could be affected by sunlight. The studies  
59 carried out in this sense involved the presence of a photosensitizer and focused on  
60 studying the influence of the formulation on the formation of photoinduced oxidized  
61 damage<sup>13</sup> while the direct absorption of UVA radiation by DNA has been widely  
62 demonstrated. So, the purpose of this work is to focus on the direct formation of  
63 oxidized lesions on DNA formulations under UVA radiation involving as cationic  
64 derivatives, a close analog of cetyltrimethylammonium bromide (CTAB), namely  
65 cetyltriethylammonium bromide (CTEAB, Figure 1).



66

67

68 **Figure 1. Structure of cetyltriethylammonium bromide (CTEAB).**

69

70 This cationic surfactant presents the advantage of being readily soluble in liquid  
71 water at any temperature ( $T_{Krafft} < 0\text{ }^{\circ}\text{C}$ ),<sup>14</sup> whereas the Krafft temperature of CTAB  
72 ( $T_{Krafft} = 25\text{ }^{\circ}\text{C}$ )<sup>14</sup> could be the source of troubles when handling it at room  
73 temperature (close to the solubility temperature). On the other hand, CTEAB  
74 presents a bulkier head group than CTAB and this affects its self-organization  
75 properties in pure water due to molecular packing considerations.<sup>15</sup> For example,  
76 although they have similar critical micelle concentrations (CMCs) in water, CTAB

77 forms elongated micelles while CTEAB rather self-assembles into much smaller,  
78 slightly ellipsoidal micelles.<sup>16,17</sup> Therefore CTEAB has been mainly used as pore  
79 directing agent for the preparation of mesoporous materials thanks to its ability to  
80 form micellar cubic phases,<sup>18–20</sup> but also as adjuvant in enhancing oil recovery  
81 technique.<sup>21,22</sup> However its interaction with DNA has not been reported yet (few  
82 authors sometimes describe the use of cetyltriethylammonium bromide in DNA  
83 extraction and purification processes, but a closer look shows that it was a  
84 misspelling of cetyltrimethylammonium bromide, which is widely used for decades in  
85 reference protocols). Therefore, it is interesting to consider this CTEAB-DNA  
86 formulation and to see whether the slight structural change (ethyl vs methyl) due to  
87 the replacement of CTAB by CTEAB could induce significant changes or not.

88 In a first part we present the self-organization properties of CTEAB in the buffer  
89 solution used as solvent for DNA. Indeed, aggregation of CTEAB has been depicted  
90 in water but the presence of electrolytes (buffer) is known to have an impact on such  
91 properties.<sup>23</sup> Therefore the determination of the CMC (by tensiometry and pyrene  
92 fluorimetry) and the depiction of the micelles (by scattering experiments) will be  
93 carried out. Then the CTEAB-DNA interactions will be characterized to check that  
94 CTEAB can also be used to formulate DNA, as an analog of CTAB. For that, the  
95 critical aggregation concentrations (CACs) will be determined by complementary  
96 techniques (tensiometry, pyrene fluorimetry, agarose gel electrophoresis, UV-Vis  
97 absorbance and circular dichroism measurements). Finally, we will investigate the  
98 effects of UVA radiation on these formulations (by agarose gel electrophoresis) and  
99 will evidence that it can led to direct DNA photosensitization. Experiments conducted  
100 in the presence of peculiar scavengers will allow a mechanistic interpretation of the  
101 results obtained.

## 2. Materials and methods

### 2.1. Materials

Triethylamine (purity 99.9 %) and 1-bromohexadecane (purity 98%) were purchased from VWR (Fontenay Sous Bois, France) and Lancaster (Morecambe, England), respectively. CTEAB was synthesized as described in the literature<sup>24</sup> and purification protocol was improved. Briefly, 1-bromohexadecane (20 g, 65 mmol) was reacted with triethylamine (10 mL, 72 mmol) in 130 mL absolute ethanol for 72 h at 85 °C (reflux). After evaporation to dryness under reduced pressure, the residue was dissolved in chloroform and ethyl acetate was added to induce precipitation. After precipitation was completed at 4 °C, the white solid was collected by filtration and washed with ethyl acetate. Final purification was achieved by solid phase extraction (SPE) on a reverse phase Chromabond C<sub>18</sub> ec chromatographic column (70 mL, 10 g) from Macherey-Nagel (first washing with water then elution with methanol). This step ensured full removal of the impurity trimethylamine hydrobromide. Evaporation of methanol afforded CTEAB as a white crystalline solid. Purity was assessed by nuclear magnetic resonance (NMR) and high-resolution mass spectroscopy (HRMS). (see *Supporting Information*).

Tris(hydroxymethyl)aminomethane (TRIS, purity 99.8 %) was from Fisher Scientific (Geel, Belgium). Supercoiled plasmid DNA (Form I) pBR322 (4361 base pairs) was purchased from Thermo Fisher Scientific (Vilnius, Lithuania), stored in  $10 \times 10^{-3}$  mol.L<sup>-1</sup> of TRIS/HCL (pH 7.6) and  $1 \times 10^{-3}$  mol.L<sup>-1</sup> of EDTA. The DNA was used after dilution in a 20 mM TRIS/HCl buffer (pH=7.4) to obtain a stock solution at [DNA-PO<sub>4</sub>] =  $4.5 \times 10^{-4}$  mol.L<sup>-1</sup>. All the DNA concentration indicated on the manuscript are in mol phosphate.L<sup>-1</sup>. Heparin sodium salt (purity 99.8%) was purchased from Alfa Aesar (Heysham, Germany) and Sodium azide (NaN<sub>3</sub>, purity 99%) from Alfa Aesar

127 (Karlsruhe, Germany). Agarose and tert-butanol ( $t\text{BuOH}$ , purity 99.5%) were obtained  
128 from Sigma-Aldrich (Saint Louis, USA), while pyrene (purity 98%) was from Acros  
129 Organics (Geel, Belgium). Ethidium Bromide (EB) was purchased as a  $10\text{ mg}\cdot\text{mL}^{-1}$   
130 aqueous solution from Invitrogen (Carlsbad, USA).  $\text{D}_2\text{O}$  (purity 99.9%) was obtained  
131 from Eurisotop (Saint Aubin, France) and T4 endonuclease V (pyrimidine dimer  
132 glycosylase) from BioLabs (New England, USA). Bromophenol blue was purchased  
133 from Sigma Aldrich (Saint Louis, USA).  
134 Water used in all experiments was produced by a two-stage Milli-Q filtration system  
135 from Millipore and had a resistivity higher than  $18.2\text{ M}\Omega\cdot\text{cm}$ .

## 136 **2.2. Surface tension measurements**

137 Surface tension measurements were performed using a Krüss EasyDyne tensiometer  
138 using the Wilhelmy plate method. Temperatures were maintained at  $25\text{ }^\circ\text{C}$  ( $\pm 0.1\text{ }^\circ\text{C}$ )  
139 by circulating thermostated water through a jacketed vessel containing the solution.  
140 For each experiment, surface tension was measured three times (each time ten  
141 readings were performed and averaged) after an equilibration time of 30 minutes.

## 142 **2.3. Fluorescence measurements**

143 A stock solution of pyrene ( $10^{-4}\text{ mol}\cdot\text{L}^{-1}$ ), was prepared in methanol and stored in  
144 glass vials at  $4\text{ }^\circ\text{C}$ . The solutions for fluorescence probing studies were prepared by  
145 adding  $1\text{ }\mu\text{L}$  of this pyrene stock solution ( $10^{-4}\text{ mol}\cdot\text{L}^{-1}$ ) to  $399\text{ }\mu\text{L}$  of the sample  
146 solution. Final concentration of pyrene was  $2.5\times 10^{-7}\text{ mol}\cdot\text{L}^{-1}$ , while the amount of  
147 methanol remained very low ( $0.25\text{ }\%$  vol.) Fluorescence measurements were  
148 performed on a Fluorolog Horiba spectrofluorimeter equipped with channel multiple  
149 tau correlator (auto and cross correlation, 1088 real time channels) and a

150 thermostated cuvette holder at  $25 \pm 0.1$  °C. Excitation wavelength was 335 nm, and  
151 the fluorescence emission was recorded between 350 and 500 nm.

## 152 **2.4. UV-Visible absorbance and circular dichroism measurements**

153 Absorption spectra were recorded on a Hewlett Packard HP 8452A Diode Array  
154 Spectrophotometer with an optical path length of 1 cm.

155 Circular dichroism spectra were obtained from a circular dichroism spectrometer J-  
156 815 from Jasco equipped with a 150 W Xenon arc lamp light source, using a  
157 bandwidth of 0.5 nm, with medium sensitivity with a maximum of 200 mdeg. Data  
158 acquisition was done in continuous mode.

## 159 **2.5. Multi-angle light scattering experiments**

160 The multi-angle light scattering (MALS) measurements were performed on a 3D LS  
161 Spectrometer from LS Instruments (Fribourg, Switzerland) equipped with a 100 mW  
162 high performance DPSS Laser (Cobolt) operating at  $\lambda = 660$  nm, a two-channel  
163 multiple tau correlator (auto and cross correlation, 1088 real time channels), a 3D  
164 cross-correlation module, a variable-angle detection system, two high sensitivity APD  
165 detectors, an automated laser attenuation system combined with on-line laser  
166 intensity measurement, a temperature controllable sample chamber with index  
167 matching vat, and an external circulator for temperature control (Julabo CF31).

168 Before analysis (and otherwise stated), samples were filtered through 0.2  $\mu\text{m}$   
169 hydrophilic PTFE filters into the cylindrical scattering cells.

170 Measurements were performed at 25°C for scattering angles  $\theta$  ranging from 12° to  
171 150°, corresponding to scattering wave vectors  $q$  in the range from  $2.7 \times 10^{-3}$  to  
172  $2.4 \times 10^{-2}$   $\text{nm}^{-1}$ . As CTEAB solutions did not produce multiple scattering, the 3D-cross

173 correlation configuration was not required and the measurement were performed in  
174 the classical 2D-pseudo cross configuration.

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

## 179 **2.6. Small-Angle X-ray Scattering experiments**

180 SAXS (Small-Angle X-ray Scattering measurements) were conducted at the  
181 European Synchrotron Radiation Facility (ESRF, Grenoble, France) at the ID02  
182 beamline. CTEAB solutions (5 to 20 mM) were transferred into glass capillaries  
183 before measurements. The sample-to-detector distance was 2.5 m. Using 1 Å X-ray  
184 wavelength, the  $q$  range covered was  $2.9 \times 10^{-3} \text{ \AA}^{-1} < q < 3.1 \times 10^{-1} \text{ \AA}^{-1}$ . For each  
185 solution, 10 scans were recorded and averaged. For each scan, exposure time was  
186 kept as low as 100 ms to prevent from radiation damage. The observed intensities  
187  $I(q)$  were corrected for transmission, solvent, and detector response. The analysis of  
188 the  $I(q)$  profiles was performed using the SasView software  
189 (<http://www.sasview.org/>).

## 190 **2.7. Irradiation experiments**

191 DNA irradiation was made with a Mercury Xenon lamp (Oriel instruments, 500W)  
192 equipped with a long pass filter  $\lambda \geq 335\text{nm}$  (Newport 20CGA-335 GC505) at 20°C.

193 The irradiated and non-irradiated sample volume was 100  $\mu\text{L}$ . The final  
194 concentrations in TRIS/HCl buffer (20 mM, pH=7.4) were  $9 \times 10^{-5} \text{ mol phosphate} \cdot \text{L}^{-1}$   
195 for the plasmid DNA pBR322, from 0 to  $4 \times 10^{-3} \text{ mol} \cdot \text{L}^{-1}$  for the CTEAB solutions,

196  $200 \times 10^{-3} \text{ mol L}^{-1}$  for  $\text{NaN}_3$  and 4% v/v for  $t\text{BuOH}$ . Each experiment was at least  
197 triplicated.

198

## 199 **2.8. Agarose gel electrophoresis**

### 200 2.8.1. Sample preparation

201 50  $\mu\text{L}$  of the sample solution were used on the T4 endonuclease V experiment. It  
202 consists to add 10  $\mu\text{L}$  of T4 endonuclease V (10000U/ml) and to incubate the solution  
203 30 minutes at  $37^\circ\text{C}$ . 5  $\mu\text{L}$  of bromophenol blue solution and 10  $\mu\text{L}$  of Heparin 20%  
204 (w/w in TRIS/ HCl buffer (20 mM, pH= 7.4) were added.

205 To the samples without T4 endonuclease V treatment, 5  $\mu\text{L}$  of bromophenol blue  
206 solution, 10  $\mu\text{L}$  of Heparin 20% (w/w in TRIS/ HCl buffer) and 10  $\mu\text{L}$  TRIS/ HCl buffer  
207 (20 mM, pH= 7.4) were added.

### 208 2.8.2 Electrophoresis and photodensitometry

209 An aliquot of 40  $\mu\text{L}$  of each sample was deposited into 0.8% agarose gel containing  
210 0.02 % of Ethidium Bromide. Electrophoresis migration was carried out for 4 hours  
211 under a constant electric field of 80 mV.

212 Single strand breaks (SSB) were quantified by photodensitometry using the ImageJ  
213 software. To correct the lower efficiency of ethidium bromide binding to Form I DNA  
214 with respect to Form II, a coefficient of 1.66 was used.<sup>25</sup>

215

216

### 3. Results and discussion

217

#### 3.1. Self-organization of CTEAB in TRIS/HCl buffer

218 CTEAB surfactant (Figure 1) was synthesized as described in the literature<sup>24</sup> and

219 dissolved in 20 mM TRIS/HCl buffer solutions at pH = 7.4. Determination of the CMC

220 by tensiometry was performed by measuring the variation of the surface tension as a

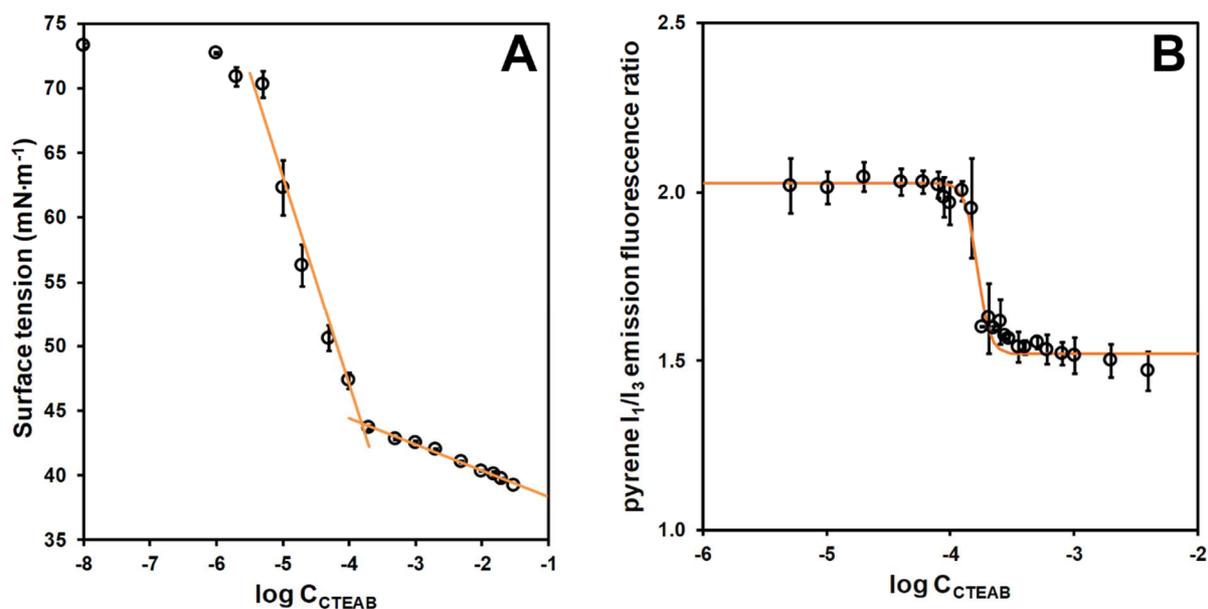
221 function of the surfactant concentration. In the dilute regime, the surface tension

222 value is close to the one of water and then it decreases when the concentration of

223 surfactant increases. At the threshold value which corresponds to the CMC, the

224 surface tension does not vary much upon increasing surfactant concentration (Figure

225 2A).



226

227 **Figure 2. Variation of the surface tension (A) and of the pyrene  $I_1/I_3$**

228 **fluorescence emission ratio (B) of CTEAB solutions as a function of the CTEAB**

229 **concentration in 20 mM TRIS/HCl buffer (pH = 7.4) at 25 °C.**

230

231 By this technique the CMC of CTEAB was found to be  $1.5 (\pm 0.2) \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$  in the

232 20 mM TRIS/HCl buffer (pH = 7.4) at 25 °C. This value is much lower than the CMC

233 values reported in the literature for CTEAB in water:  $7\text{-}8 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ , as determined  
 234 from tensiometry and conductivity experiments.<sup>16,17,26–29</sup> This decrease of CMC  
 235 means that the aggregation is favored in TRIS/HCl buffer compared to pure water.  
 236 The effect of the electrolyte is to partially screen the repulsion electrostatic  
 237 interactions between the similar charged head groups of the surfactant and therefore  
 238 to promote aggregation. Thereby, the CMC is lowering by addition of electrolyte.  
 239 In the same way, Haq, Z. and coworkers<sup>30</sup> reported a decrease of the CMC of the  
 240 CTAB from  $0.98 \text{ mol}\cdot\text{L}^{-1}$  (at 303 K) to  $0.90 \text{ mol}\cdot\text{L}^{-1}$  at  $3 \text{ mol}\cdot\text{L}^{-1}$  of NaCl, indicating the  
 241 significant role of the electrostatic interactions between surfactant and electrolyte.

242 From the tensiometry curve, one can also estimate the minimal molecular cross-  
 243 sectional area at the air/water interface  $A_{s,min}$  at the CMC.<sup>23</sup> This is done using the  
 244 Gibbs equation which relies the maximal surface excess concentration  $\Gamma_{max}$  to the  
 245 slope of the surface tension vs log C curve before the CMC as follows:

$$\Gamma_{max} = -\frac{1}{2.303nRT} \cdot \frac{\partial\gamma}{\partial\log C} \quad (1)$$

246 where T is the temperature, R the gas constant, C the concentration, and n the  
 247 number of adsorbed species (here  $n = 2$ , accounting for CTEA<sup>+</sup> and Br<sup>-</sup>). Then  $A_{s,min}$   
 248 can be expressed as (2):

$$A_{s,min} = \frac{1}{N_A\Gamma_{max}} \quad (2)$$

249 where  $N_A$  is the Avogadro number. Applying this to the curve in Figure 2A gives  
 250  $A_{s,min} = 110 (\pm 10) \text{ \AA}^2\cdot\text{molecule}^{-1}$ . This value is quite high compared to a surface of  
 251  $62 \text{ \AA}^2\cdot\text{molecule}^{-1}$  reported for CTEAB in pure water,<sup>29</sup> suggesting that the distance  
 252 between the surfactants got increased at the interface in the presence of electrolytes.  
 253 The fact that the interface occupation appears less compact (and the surfactants

254 bulkier) might be due to the accumulation of species (like water or ions) between the  
255 surfactants.

256 The aggregation properties of CTEAB in TRIS/HCl buffer have also been  
257 studied by pyrene fluorescence measurements. Pyrene is a hydrophobic fluorescent  
258 probe that has been widely used for the characterization of self-assembling systems  
259 due to the specific variation of its emission fluorescence as a function of the local  
260 polarity.<sup>31</sup> In its specific multi-peak emission spectrum, the  $I_1/I_3$  intensity ratio between  
261 the first and third peaks ( $I_1$  at 372 nm and  $I_3$  at 383 nm respectively) is decreasing if  
262 the polarity decreases. In surfactant systems, this  $I_1/I_3$  ratio varies as a function of the  
263 surfactant concentration with a sharp decrease at the CMC, from a high value (below  
264 CMC, pyrene located in water) to a lower one (above CMC, pyrene located in the  
265 hydrophobic inner core of the micelles). The results regarding pyrene fluorescence as  
266 a function of CTEAB concentration in TRIS/HCl buffer are represented in Figure 2B.  
267 The variation of the  $I_1/I_3$  ratio has been adjusted by a sigmoidal Boltzmann function to  
268 determinate the CMC.<sup>32</sup>

269 From this fluorescence measurements, the aggregation of CTEAB in TRIS/HCl  
270 buffer occurs at  $CMC = 1.6 (\pm 0.2) \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ , which is in good accordance with  
271 tensiometry measurements (using this quick technique we also checked the CMC of  
272 CTEAB in pure water and found  $7.1 (\pm 0.2) \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ , which is very close to the  
273 values reported in the literature,<sup>16,17,26–29</sup> see *Supporting Information, Figure S3*). In  
274 addition, the intrinsic value of the  $I_1/I_3$  ratio is directly related to local micropolarity: a  
275 low ratio value ( $<1$ ) indicates that pyrene is surrounded by a nonpolar environment,  
276 while higher ratio values ( $>1$ ) show that the pyrene environment is polar.<sup>31</sup> This ratio  
277 has been shown to vary from about 0.6 in alkanes to 1.2-1.6 in alcohols and up to 1.9  
278 in water.<sup>31,33</sup> In the present case of CTEAB, we observed a decrease of the  $I_1/I_3$  ratio

279 from 2.0 to 1.5 at the CMC. This shows that even when located in micelles, the  
280 pyrene probe is still in a rather polar environment. This suggest that water molecules  
281 have partially penetrated the inner micellar cores, leading to an increase of the  $I_1/I_3$   
282 ratio.<sup>31,34</sup> This is directly related to the surfactants head groups: bulky heads induce  
283 less compact interfaces and allow penetration of water, which is here consistent with  
284 large polar surface areas determined for CTEAB from tensiometry measurements.

285 In order to get more insights on the structure of the aggregates formed by  
286 CTEAB in aqueous TRIS/HCl solutions, both multi-angle static- and dynamic light  
287 scattering (MASLS and MADLS) experiments were performed at different  
288 concentrations ( $[CTEAB] = 5$  to  $30 \times 10^{-3}$  mol·L<sup>-1</sup>). Small angle X-Ray scattering  
289 (SAXS) measurements were also carried out.

290 In light scattering experiments, the scattering wave vector  $q$  is defined as a function  
291 of the scattering angle  $\theta$  as described by equation (3).

$$q = \frac{4\pi n \sin(\theta/2)}{\lambda} \quad (3)$$

292 where  $n$  is the refractive index of the medium and  $\lambda$  the wavelength of the incident  
293 light.

294 In MASLS, the excess of scattered intensity by a sample with respect to the  
295 solvent is converted into absolute intensity (Rayleigh ratio  $R_\theta$ , in cm<sup>-1</sup>) using toluene  
296 as a reference (see *Supporting Information*). Absolute Rayleigh excess ratios of  
297 CTEAB solutions were measured in the  $2.6 \times 10^{-4}$ - $2.5 \times 10^{-3}$  Å<sup>-1</sup>  $q$  range. In all cases,  
298 flat plateaus were obtained meaning that the scattering was isotropic and that the  
299 aggregates formed by CTEAB were very small in size (see *Supporting Information*,  
300 *Figure S4*). As a consequence, the radius of gyration  $R_g$  could not be determined due  
301 to the  $q$ -independent form factor in this  $q$  range. However, the variation of the  
302 Rayleigh ratio as a function of the CTEAB concentration could allow Debye analysis

303 (see *Supporting Information, Figure S5*). Therefore, the apparent mass of the  
 304 aggregates was determined to be  $M_w = 41.1 \pm 1.6$  kDa. From this value and using the  
 305 molar weight of CTEAB, it was possible to calculate the aggregation number ( $N_{agg}$ )  
 306 which was found to be  $N_{agg} = 101 \pm 4$ . This value is larger or equal to those reported  
 307 for CTEAB in pure water ( $N_{agg} = 49-100$ ),<sup>17,29</sup> confirming that micellization in TRIS  
 308 buffer is favored and allows a larger number of surfactant molecules to aggregate.

309 What is measured experimentally in MADLS is the autocorrelation function  
 310  $g^{(2)}(q, \tau)$  of the scattered intensity  $I(q, t)$ , normalized over time (4):

$$g^{(2)}(q, \tau) = \frac{\langle I(q, t)I(q, t + \tau) \rangle}{\langle I(q, t) \rangle^2} \quad (4)$$

311 This latter is also related to the correlation function of the electric field  $g^{(1)}(q, \tau)$  by  
 312 the Siegert relationship (5):

$$g^{(2)}(q, \tau) = 1 + \beta |g^{(1)}(q, \tau)|^2 \quad \beta \leq 1 \quad (5)$$

313 For monodisperse spherical particles under Brownian motion, the autocorrelation  
 314 function decays exponentially over time (6):

$$g^{(1)}(q, \tau) = e^{-\Gamma\tau} \quad (6)$$

315 where  $\Gamma = D_0 q^2$  is the decay rate and  $D_0$  is the translational diffusion coefficient of the  
 316 particles. The hydrodynamic radius of equivalent sphere ( $R_h$ ) is then obtained by the  
 317 Stokes-Einstein relationship:

$$R_h = \frac{kT}{6\pi\eta D_0} \quad (7)$$

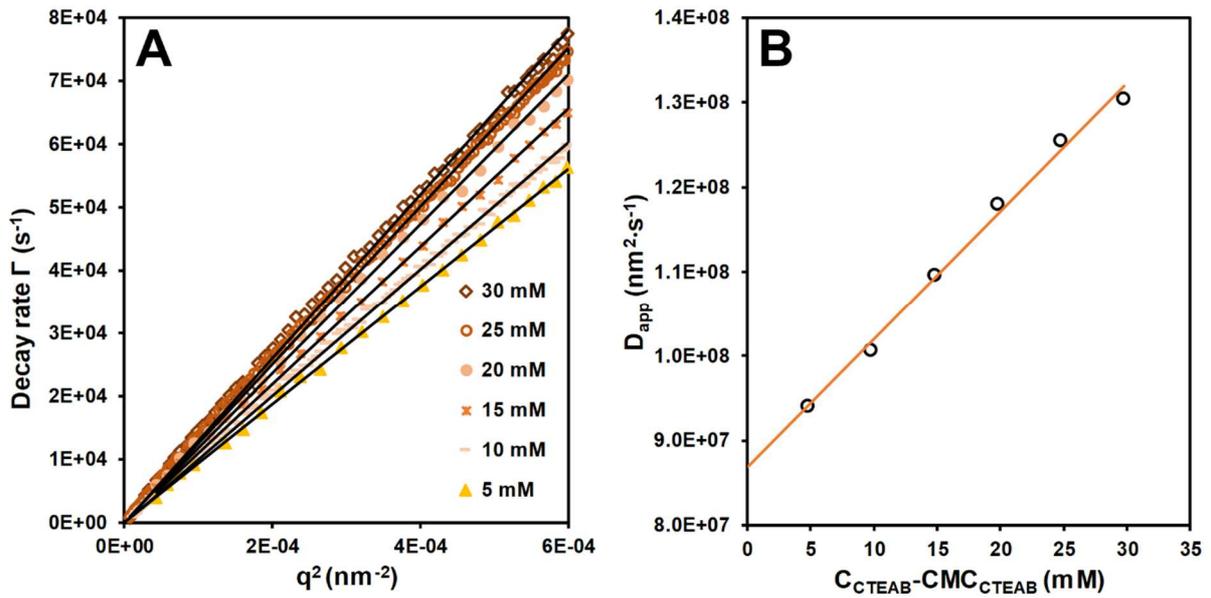
318 where  $k$  is the Boltzmann constant,  $T$  the absolute temperature, and  $\eta$  the solvent  
 319 viscosity.

320 Experimentally, simple scattering was always observed (no multiple scattering) from  
 321 the clear, transparent CTEAB solutions. In all cases, the autocorrelation functions  
 322 were well fitted using a Cumulant analysis<sup>35</sup> (see *Supporting Information, Figure S6*),

323 affording the intensity-weighted decay rates  $\Gamma$ . Plotting decay rates as a function of  $q^2$   
 324 afforded linear profiles  $\Gamma = D_{app}q^2$  (Figure 3A), meaning that single relaxation  
 325 mechanisms were observed associated to single apparent translational diffusion  
 326 coefficients ( $D_{app}$ ).

327

328



329

330 **Figure 3. Linear variations of decay rates as a function of  $q^2$  (A). Variation of the**  
 331 **apparent diffusion coefficient  $D_{app}$  as a function of the micelle concentration**  
 332 **(B).**

333

334 The apparent diffusion coefficients showed a linear dependence as a function of the  
 335 micelle concentration (*ie*  $C_{CTEAB} - CMC_{CTEAB}$ ) (Figure 3B):

$$D_{app} = D_0 + k(C_{CTEAB} - CMC_{CTEAB}) \quad (8)$$

336 This reflects inter-particle interactions (probably electrostatic repulsions between  
 337 positively charged aggregates of cationic surfactants). Extrapolation at infinite dilution  
 338 afforded  $D_0 = 8.68 \times 10^7 \text{ nm}^2 \cdot \text{s}^{-1}$  and  $R_{h,0} = 2.83 \text{ nm}$  from the Stokes-Einstein

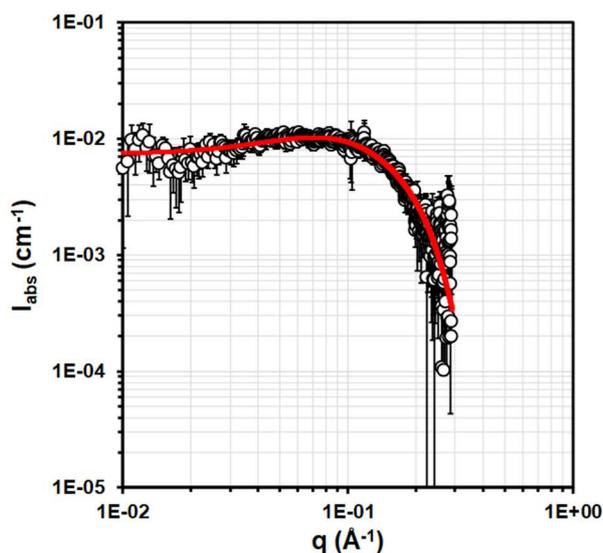
339 relationship. This hydrodynamic radius is comparable to the dimensions of micelles  
340 formed by CTEAB in pure water.<sup>16,17</sup>

341 Knowing the radius of the micelles, the determination of their surface gives  
342 101 nm<sup>2</sup>, and dividing it by the aggregation number, the surface occupied by one  
343 surfactant head group is 1 nm<sup>2</sup>, which is consistent with the surface estimated from  
344 tensiometry measurements. It was also possible to determine the volume occupied  
345 per surfactant at 0.94 nm<sup>3</sup>. The comparison of this value to the molecular volume of  
346 CTEAB (0.64 nm<sup>3</sup>) indicates that about 10 water molecules are present around each  
347 CTEAB molecule, confirming that CTEAB micelles are highly hydrated.

348 Complementary SAXS experiments were also carried out to get more  
349 information on CTEAB micelles. The general expression of the scattered intensity  
350  $I(q)$  is:

$$I(q) = \Phi \cdot V \cdot (\Delta\rho)^2 \cdot P(q) \cdot S(q) \quad (9)$$

351 where  $q = [4\pi \sin(\theta/2)]/\lambda$  is the scattering vector ( $\theta$  is the scattering angle),  $\Phi$  is the  
352 volume fraction of the scatterers,  $V$  is their volume, and  $\Delta\rho$  is their difference in  
353 scattering length density (SLD) with respect to the solvent.  $P(q)$  is the scatterers'  
354 form factor for which analytical expressions have been reported for various  
355 geometries.<sup>36,37</sup>  $S(q)$  is the structure factor accounting for inter-particle interactions,  
356 for which different models are also available.<sup>36,37</sup> Regarding the CTEAB solutions, the  
357 scattering intensities were very low, indicating a weak electronic contrast between the  
358 solvent and the aggregates. Moreover, the profiles of the scattering curves suggested  
359 a spherical shape for the aggregates, as illustrated in Figure 4 (full curves and fitting  
360 parameters are reported in the *Supporting Information*).



361  
 362 **Figure 4. SAXS profile of 20 mM CTEAB solution in 20 mM TRIS/HCl (pH = 7.4)**  
 363 **at 25 °C. The solid line represents the adjustment of the data with a spherical**  
 364 **form factor and a structure factor of a charged hard sphere.**

365  
 366 Indeed, the data were efficiently adjusted using a  $P(q)$  spherical model, whereas the  
 367 ellipsoidal model used by others to describe CTEAB micelles in pure water was not  
 368 convenient here.<sup>16,17</sup> In addition, a structure factor  $S(q)$  was included, which  
 369 corresponded to a charged hard sphere model to take into account electrostatic  
 370 repulsions between positively charged micelles (visible at low  $q$  as a decline of  $I(q)$  in  
 371 the SAXS curve). However, the classical core-shell structure could not be evidenced  
 372 contrary to analogous cationic surfactants.<sup>38</sup> Instead, the SAXS profiles rather  
 373 corresponded to small spherical shapes ( $R = 14 \text{ \AA}$ ) with a scattering contrast that  
 374 was the one of pure hydrophobic alkyl chains. This indicates that only a part of the  
 375 inner core of the micelles could be observed by this technique, and it is again in  
 376 accordance with a possible penetration of water towards the inner, “hydrophobic”  
 377 core of the aggregates.

### 3.2. Interaction between CTEAB and DNA

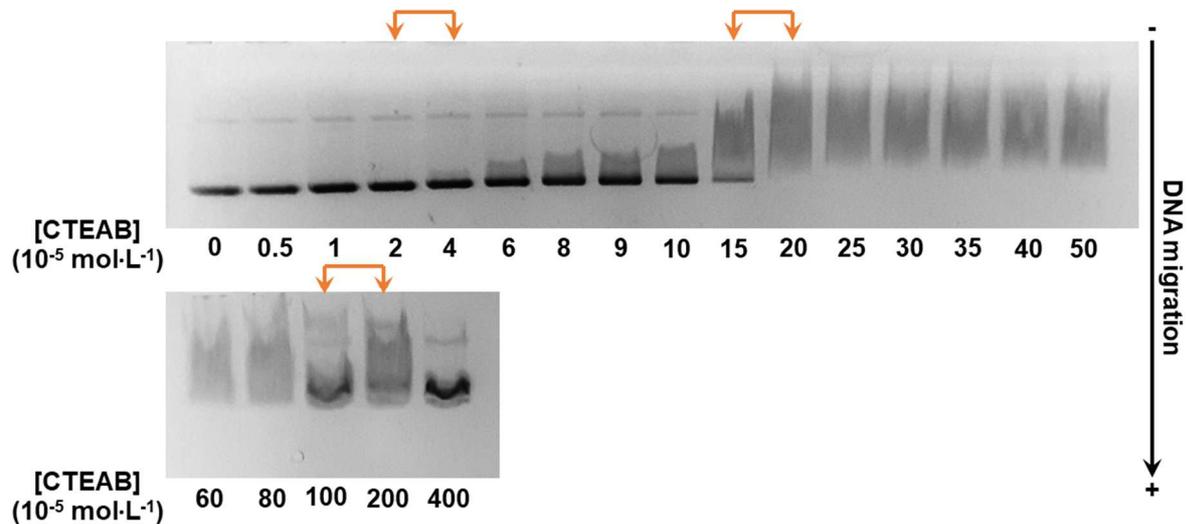
Cationic surfactants have been considered for a few decades as potential non-viral vectors for gene delivery, thanks to their ability to easily form complexes with DNA. This complexation induces the formation of highly organized assemblies, involving various intermolecular interactions (electrostatic, hydrophobic, etc.) and highly cooperative binding.<sup>39,40</sup> In this field, CTAB was one of the most studied cationic surfactants. It has been shown that combination of electrostatic and hydrophobic interactions between CTAB and DNA induces functional and morphological changes of the biopolymer, particularly DNA compaction.<sup>41-53</sup> Considering CTEAB as a potential non-viral vector, it is therefore important to characterize the interactions occurring between the surfactant and the nucleic acid involved in such DNA formulations. It is especially interesting to study the effect of replacing CTAB by CTEAB on the different phase transitions, as we have shown that these structurally close surfactants differ, however, in their micellization properties.

Three macroscopic states were observed on CTEAB-DNA solutions depending on the CTEAB concentration. For  $[\text{CTEAB}] < 20\text{-}30 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$  clear solutions were observed. Between  $20\text{-}30 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$  and  $100\text{-}200 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ , turbid solutions appear. For higher CTEAB concentrations ( $[\text{CTEAB}] > 100\text{-}200 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ ), turbid solutions turned to clear.

Therefore, complementary techniques (agarose gel electrophoresis, tensiometry, fluorimetry, UV-Visible spectrometry and circular dichroism) were used to study the binary system made of CTEAB and the supercoiled plasmid DNA pBR322 at various molar ratios.

401

402 Agarose gel electrophoresis was first carried out to study CTEAB-DNA  
403 interactions with CTEAB concentrations ranging from 0 to  $4 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$  (Figure 5).

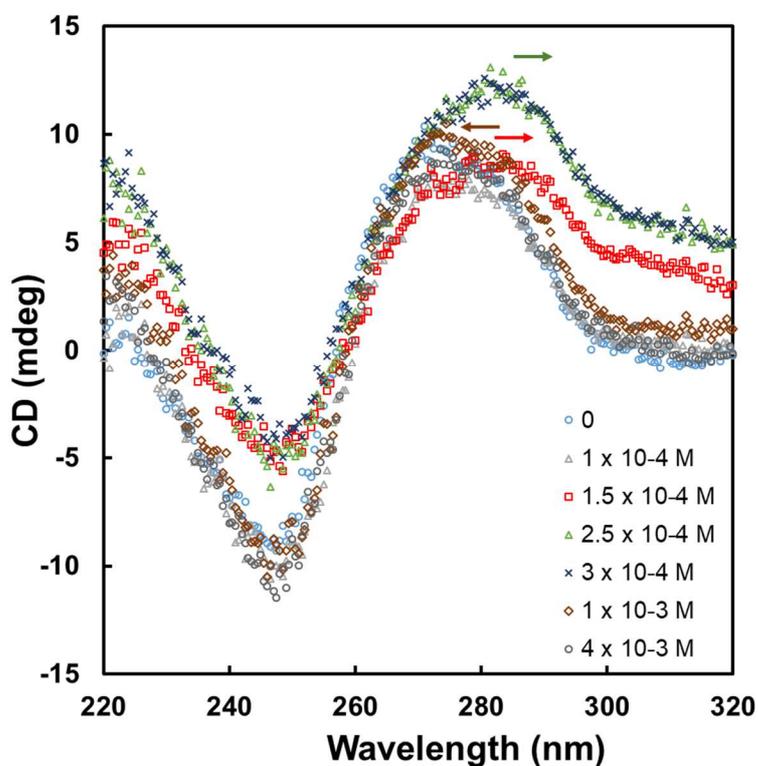


404 **Figure 5. Electrophoresis agarose gel of plasmid DNA in the presence of**  
405 **various CTEAB concentrations (DNA concentration was  $[\text{DNA-PO}_4] = 9 \times 10^{-5}$**   
406  **$\text{mol}\cdot\text{L}^{-1}$ ). Orange arrows indicate phase transitions.**

407

408 Analysis of the electrophoresis agarose gel indicates the existence of different  
409 CTEAB-DNA interactions. At low CTEAB concentration, below  $4 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$   
410 ( $[\text{CTEAB}]/[\text{DNA}] = 0.44$ ), the cooperative binding between DNA and CTEAB only  
411 slightly impacts the DNA mobility. In the second region, for  $2-4 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1} <$   
412  $[\text{CTEAB}] < 15-20 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ , ( $0.44 < [\text{CTEAB}]/[\text{DNA}] < 2.2$ ) the complexation of  
413 CTEAB to DNA reduces the electrophoresis mobility of the DNA and a smear  
414 appears on the gel.<sup>13</sup> This results from the formation of aggregates between DNA  
415 and CTEAB, leading to the gradual compaction of DNA. Above  $15-20 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$   
416 ( $[\text{CTEAB}]/[\text{DNA}] = 2.2$ ), the CTEAB-DNA compaction seems complete as it is no  
417 longer possible to observe the initial DNA band on the agarose gel. The

418 corresponding solutions appear slightly turbid. For CTEAB concentrations above  
419  $100 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$  ( $[\text{CTEAB}]/[\text{DNA}] = 11$ ), the electrophoretic mobility of DNA seems to  
420 increase, with the re-appearance of a not-so-well defined band. This could indicate the  
421 formation of another type of CTEAB-DNA complex in the presence of a large excess  
422 of CTEAB and notably a decompaction process of DNA. In the literature, this  
423 process was ascribed to the presence of an excess of the cationic surfactant, which  
424 promotes the DNA decompaction.<sup>47,54,55</sup> To go further into the understanding of the  
425 interaction mechanisms between DNA and CTEAB, circular dichroism spectroscopy  
426 (CD) was realized. The DNA circular dichroic spectrum exhibits two peaks. The first  
427 one is a negative band at 245 nm, due to the helicity of DNA. The second one, at 278  
428 nm is a positive band corresponding to the base stacking, as shown in Figure 6.<sup>56-59</sup>



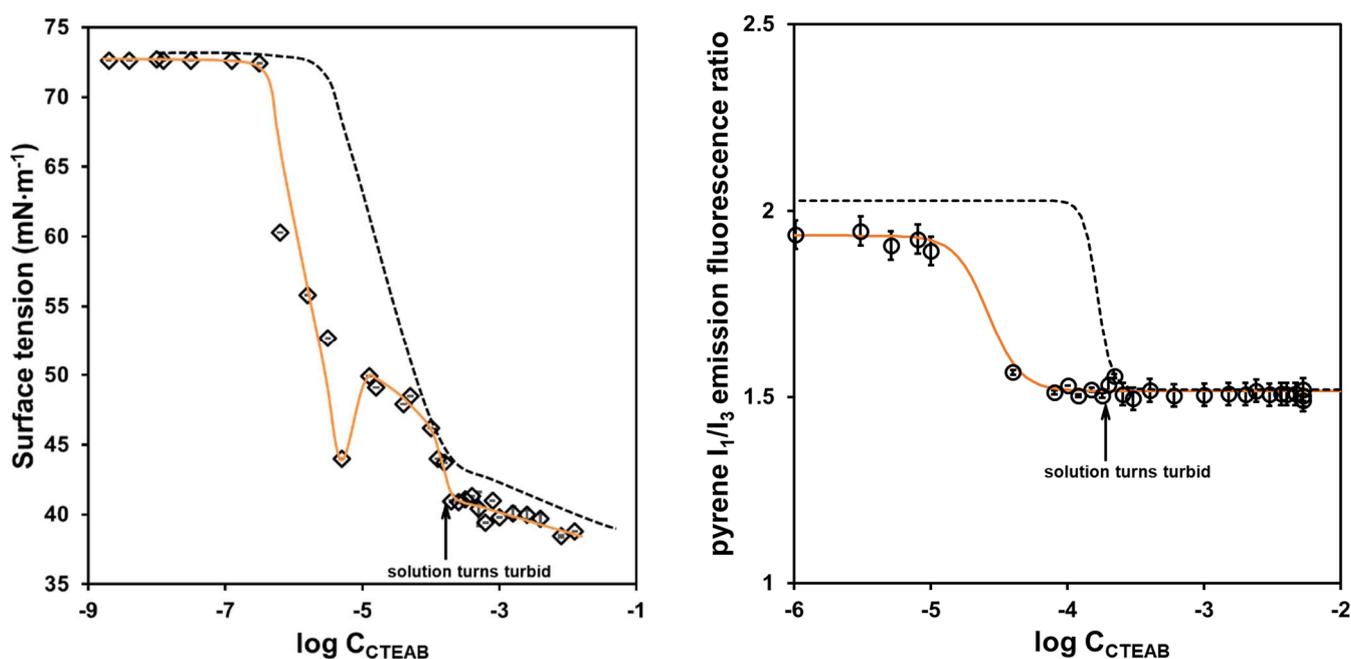
429  
430 **Figure 6. Circular dichroism spectra in CTEAB-DNA solutions for various**  
431 **CTEAB concentrations ( $[\text{DNA-PO}_4^-] = 9 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ , 20 mM TRIS/HCl buffer, pH**  
432 **= 7.4) at 25 °C.**

433

434 The CTEAB-DNA complexes, in the two phase regions, present a red-shift of the  
435 positive signal from 278 nm to 284 nm, for  $[CTEAB] > 15 \times 10^{-5} \text{ mol.L}^{-1}$ . This suggests  
436 a minor changing of interactions between the DNA bases and the evidence of the  
437 DNA compaction process in presence of CTEAB surfactant.<sup>58</sup> The observed  
438 transition could be due by the reduction of the DNA hydration layer, caused by a  
439 helical enhancement and a pitch shortening.<sup>58,59</sup> With further CTEAB concentration  
440 increase (from  $100 \times 10^{-5} \text{ mol.L}^{-1}$ ), there is a blue shift of the positive band and the  
441 position and intensity of that band recovers as DNA bands. It shows that CTEAB can  
442 decompact DNA with excessive amount of surfactant as it was already described in  
443 the literature for other surfactants.<sup>47,54,55</sup> These two peculiar CTEAB concentrations  
444 are on the same range that the ones find with the electrophoresis experiment.

445

446 CTEAB-DNA interactions were also evidenced by surface tension  
447 measurements. Figure 7A shows the variation of the surface tension of the DNA  
448 solution as a function of the CTEAB concentration.



449 **Figure 7. Variation of the surface tension (A), and of the pyrene  $I_1/I_3$**   
450 **fluorescence emission ratio (B) of CTEAB solutions as a function of CTEAB**  
451 **concentration, in the presence of DNA ( $[DNA-PO_4^-] = 9 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ , 20 mM**  
452 **TRIS/HCl buffer, pH = 7.4) at 25 °C. Dashed lines refer to CTEAB alone, for**  
453 **comparison.**

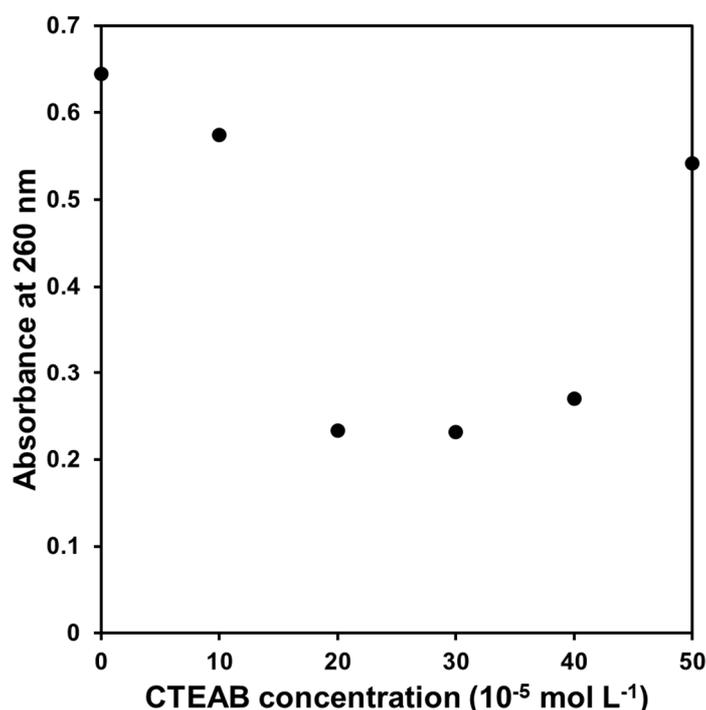
454

455 The curve profile obtained in the presence of DNA is more complex than the  
456 one for surfactant alone. Indeed, surface tension started to decrease at very low  
457 CTEAB concentrations ( $< 10^{-6} \text{ mol}\cdot\text{L}^{-1}$ ) to reach a first minimum at  $[CTEAB] =$   
458  $0.16 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$  ( $[CTEAB]/[DNA] = 0.02$ ). It occurs well below the CMC of CTEAB,  
459 meaning that this phenomenon does not correspond to the formation of micelles. This  
460 rather shows the synergistic interaction between oppositely charged species that  
461 leads to the formation of highly surface active CTEAB-DNA complexes located at the  
462 air/water interface, as proposed by others.<sup>41,60–62</sup> This complexation is driven both by  
463 electrostatic interactions but also by hydrophobic interactions between the surfactant  
464 and the biopolymer.<sup>41</sup> In this concentration range, the CTEAB molecules are believed  
465 to gradually decorate the DNA strand by progressive charge neutralization. Then,  
466 upon increasing CTEAB concentration, the surface tension rises to a local maximum  
467 and finally decreases again and reaches the curve of pure CTEAB. This could be  
468 attributed to a partial desorption of the CTEAB-DNA complexes from the interface as  
469 they accumulate in the bulk solution upon adsorption of further surfactant, potentially  
470 forming new types of aggregates. Finally, the transition to a “plateau” coincides with  
471 the apparition of a turbid solution and is observed at  $[CTEAB] = 20 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$   
472 ( $[CTEAB]/[DNA] = 2.2$ ). At this point the polyanion is saturated with surfactant and  
473 the complex formed corresponds to formulated DNA. As the air/water interface is now

474 only occupied by surfactants, surface tension monitors then only the micellization of  
475 CTEAB upon further addition of surfactant.

476 In a complementary way, pyrene fluorescence was studied at different  
477 [CTEAB]/[DNA] ratios as previously conducted for CTEAB alone. In Figure 7B is  
478 presented the variation of the pyrene  $I_1/I_3$  emission fluorescence ratio as a function of  
479 the CTEAB concentration in a DNA solution. We observed a classical sigmoidal  
480 shape referring to a phase transition and the formation of hydrophobic domains.  
481 Below the phase transition, the  $I_1/I_3$  ratio was found to be 1.93 versus 2.03 in the  
482 case of pure CTEAB. This slight decrease means that, even if pyrene is largely in  
483 contact with water or in polar environment, it has found some more hydrophobic  
484 places to locate. Indeed, it is known that pyrene can interact with DNA *via* partial  
485 intercalation at the grooves.<sup>63,64</sup> Then the phase transition occurred at [CTEAB] =  
486  $2.8 \times 10^{-5} \text{ mol} \cdot \text{L}^{-1}$  ([CTEAB]/[DNA] = 0.31). This critical concentration is much lower  
487 than the CMC of pure CTEAB and refers here to a critical aggregation concentration  
488 (CAC) evidencing again the synergistic aggregation of CTEAB and DNA. This point is  
489 believed to refer to the transition between a state where DNA is decorated by  
490 individual surfactant molecules (neutralization phase) and a state where CTEAB  
491 molecules form local hydrophobic domains on the DNA strand (compaction phase).  
492 Pyrene can locate in these aggregates and the  $I_1/I_3$  ratio drops to the 1.51 value,  
493 which is almost identical to what observed with CTEAB alone (1.50). This means that  
494 pyrene still remains in a quite hydrated environment like in the case of CTEAB  
495 micelles, where bulky head groups allow penetration of water. Note that the limited  
496 decrease of the pyrene  $I_1/I_3$  ratio does not allow the detection of further transitions as  
497 observed for other DNA-cationic surfactant systems.<sup>65,66</sup> The steric hindrance of the  
498 triethylammonium moiety could also explain why the transition occurs at a

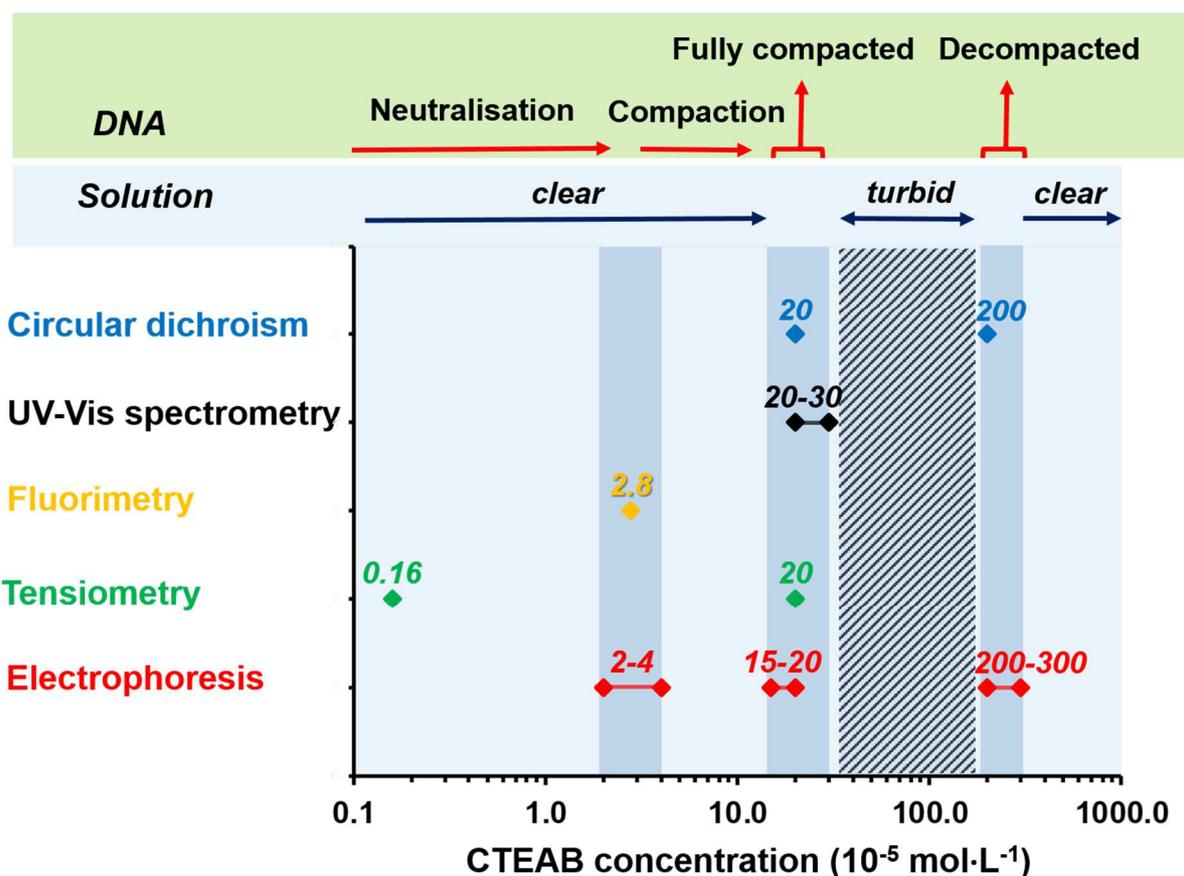
499 [CTEAB]/[DNA] ratio which is much lower than in the case of CTAB (0.31 vs. about  
500 0.70)<sup>48</sup>. Indeed, the neutralization phase is limited by the available space along the  
501 DNA strand and depends on the size of the complexing cationic surfactant.  
502 The effect of the surfactant on DNA compaction can also be followed by UV-Vis  
503 spectrometry. Thus, DNA adsorption is performed at 260 nm for different CTEAB  
504 concentrations (Figure 8). It can be observed that absorption of CTEAB-DNA  
505 complex sharply decreases until a CTEAB concentration corresponding to  $20\text{-}30 \times 10^{-5}$   
506  $\text{mol}\cdot\text{L}^{-1}$ . The increase in turbidity is due to strong electrostatic interactions between  
507 the DNA and the surfactant. This result suggests that DNA is fully compacted.  
508 CTEAB surfactant induces the reduction of the repulsive forces between the  
509 phosphate groups of DNA leading to the formation of CTEAB-DNA aggregates.<sup>56,57</sup>



510  
511 **Figure 8. Variation of the absorbance of CTEAB-DNA solutions for various**  
512 **CTEAB concentrations at 260 nm ( $[\text{DNA-PO}_4] = 9 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ , 20 mM TRIS/HCl**  
513 **buffer, pH = 7.4) at 25 °C**

514

515 As the CTEAB concentration increases above  $40 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ , the absorbance value  
 516 tends to recover to the origin value, ascribed to the decompaction process of DNA.  
 517 The combination of these five complementary techniques has allowed to evidence  
 518 different phase transitions for the CTEAB-DNA system depending on the surfactant  
 519 concentration. They are summarized in Figure 9.  
 520



521  
 522 **Figure 9. CTEAB concentrations ( $10^{-5} \text{ mol}\cdot\text{L}^{-1}$ ) at which different phase**  
 523 **transitions were evidenced by five complementary techniques in the CTEAB-**  
 524 **DNA system (DNA concentration was  $[\text{DNA-PO}_4] = 9 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$  in 20 mM**  
 525 **TRIS/HCl, pH = 7.4) at 25 °C.**

526  
 527 The first transition occurring at very low concentration has been highlighted by  
 528 surface tension measurements, and corresponds to the neutralization

529 (“lipophilization”) of DNA by CTEAB. Next, agarose gel electrophoresis and  
530 fluorimetry showed a phase transition towards a gradual compaction of DNA by  
531 CTEAB. The end of this process, leading to a fully compacted DNA was evidenced  
532 both by circular dichroism, UV-Vis spectroscopy, agarose gel electrophoresis and  
533 surface tension measurements, as well as by macroscopic observations (solutions  
534 became turbid). Finally, DNA decompaction was observed macroscopically and  
535 highlighted by circular dichroism and agarose gel electrophoresis (DNA  
536 redissolution).

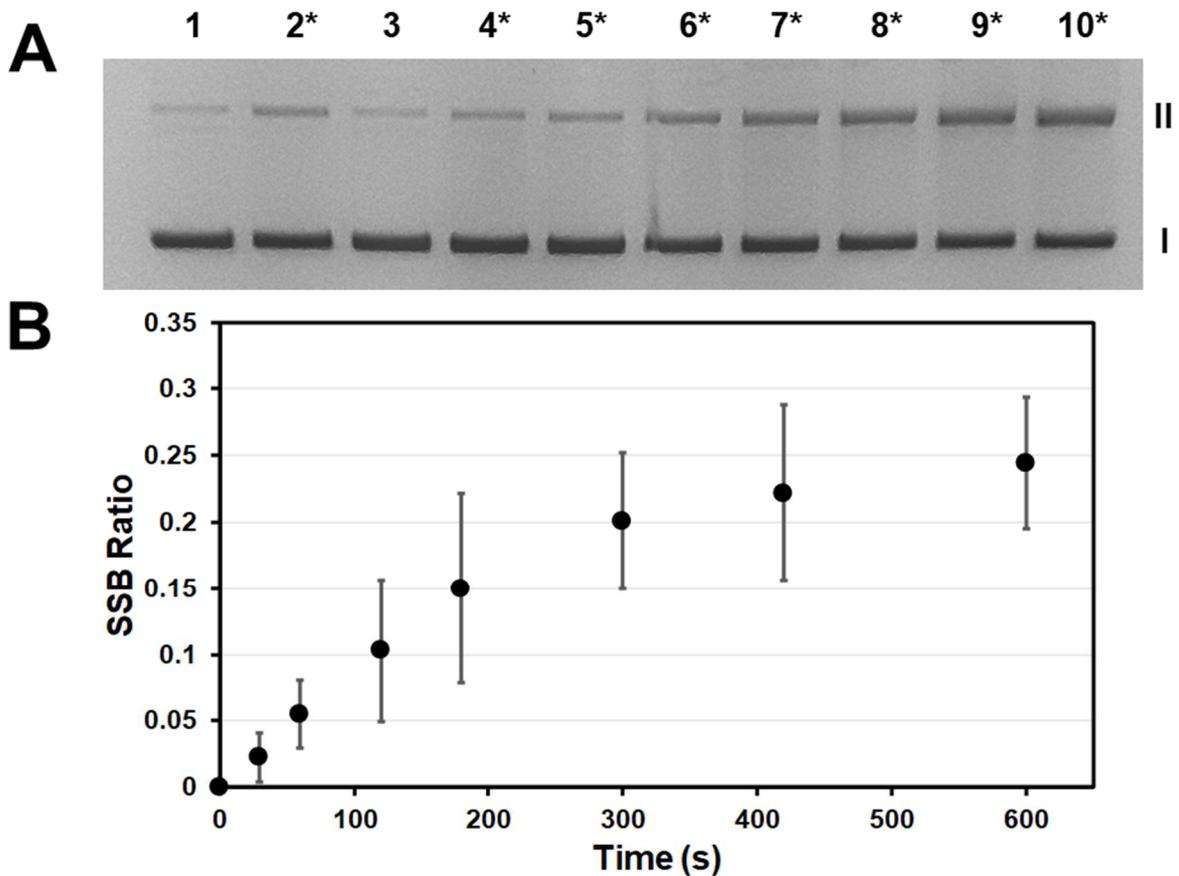
537 The formulation of DNA was then studied under UVA radiation, in order to evaluate  
538 the influence of compaction on DNA photodamage formation.

### 539 **3.3. Photostability of formulated DNA under UVA radiation**

540 Supercoiled pBR322 plasmid DNA was used as a DNA model to study the effect  
541 of CTEAB surfactant on DNA damage under UVA radiation ( $\lambda \geq 335$  nm).  
542 Photolesions such as single-strand breaks (SSB) damage can be easily revealed by  
543 agarose gel electrophoresis. Indeed, conversion of pBR322 plasmid DNA from the  
544 supercoiled form (or Form I) to the circular form (or Form II) gives two distinctive  
545 bands that can be separated and visualized by agarose gel electrophoresis (further  
546 noted as **I** and **II** on the gel pictures, respectively), and quantified by  
547 photodensitometry.

548 A first study was performed to show the effect of the irradiation time on the DNA  
549 damage in the presence of CTEAB. On figure 10A, electrophoresis agarose gel  
550 shows the DNA damage for irradiation times varying from 30 seconds to 10 minutes.  
551 A progressive increase of single strand breaks (SSB) was observed with the increase  
552 of the irradiation time. The quantification of the SSB ratios by photodensitometry  
553 (Figure 10B) shows a linear increase of the SSB ratio for the shortest irradiation

554 times and its slow-down after 200 seconds to reach a SSB ratio of about 0.25 for 10  
555 min irradiation.



556

557 **Figure 10. DNA cleavage for different irradiation times at  $\lambda \geq 335\text{nm}$  and  $20\text{ }^\circ\text{C}$  (\***  
558 **indicate the irradiated samples). A: Electrophoresis agarose gel of plasmid**  
559 **pBR322 ( $9 \times 10^{-5}\text{ mol}\cdot\text{L}^{-1}$ ) with or without CTEAB ( $5 \times 10^{-4}\text{ mol}\cdot\text{L}^{-1}$ ) in 20 mM**  
560 **TRIS/HCl buffer (pH = 7.4). Lane 1: DNA,  $t = 0\text{ s}$ ; lane 2\*: DNA,  $t = 10\text{ min}$ ; lanes**  
561 **3\* to 10\*: DNA with CTEAB,  $t = 0\text{ s}$ , 30 s, 1 min, 2 min, 3 min, 5 min, 7 min, and**  
562 **10 min. B: Single strand break (SSB) ratio for different irradiation times as**  
563 **quantified by photodensitometry.**

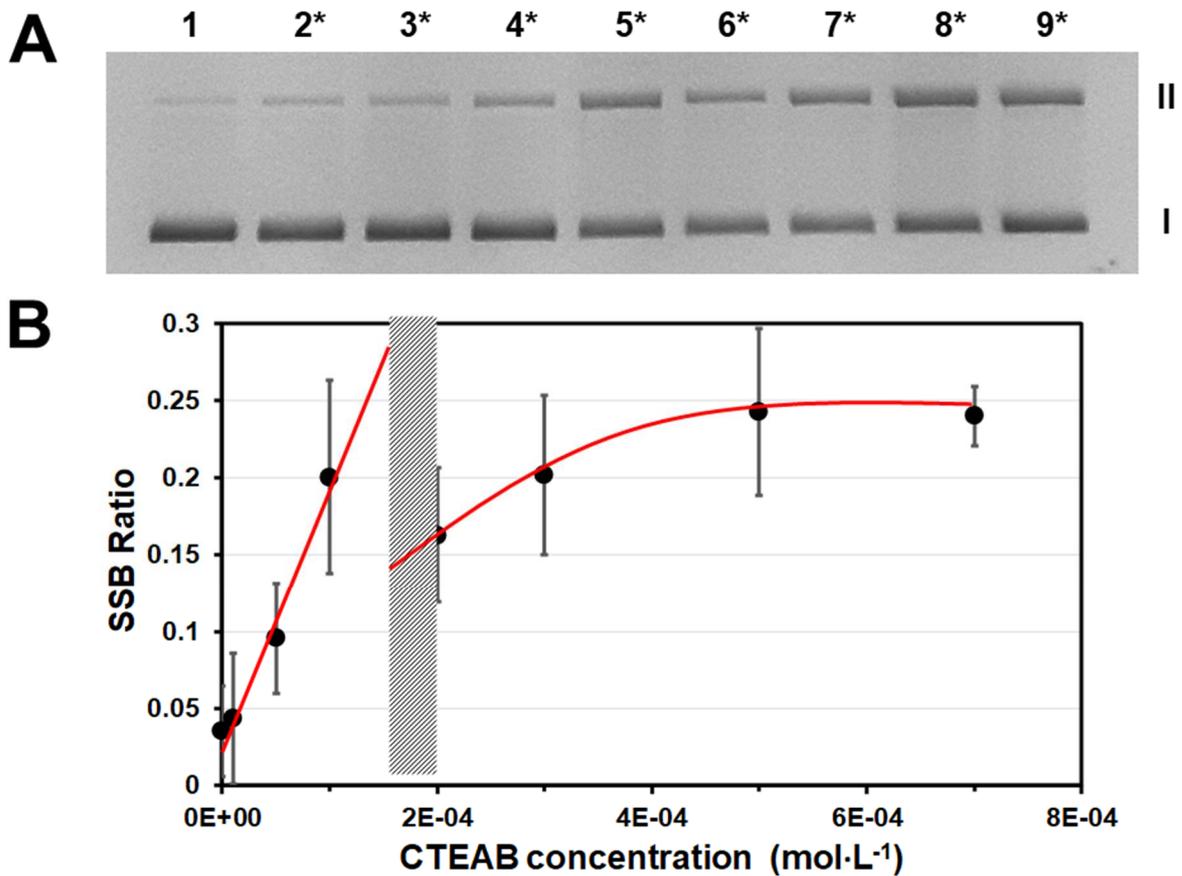
564

565 No longer time of irradiation was used because of the appearance of secondary DNA  
566 strand breaks and the formation of pBR322 plasmid DNA linear form (data not show).

567 Control UVA irradiation of DNA alone also shows SSB damage that increase with  
568 irradiation time, but twice less than in the lipoplex (see agarose gel and  
569 photodensitometry quantification in the *Supporting Information*). DNA damage by  
570 UVA light were already described in the literature and different hypothesis were made  
571 to explain how DNA can be cleaved by UVA radiation whereas the nucleotides do not  
572 absorb at these wavelengths.<sup>11,67-69</sup> It has been shown that DNA solution presents a  
573 slight and broad absorbance band in UVA range which could be enough to induce  
574 UVA deterioration of DNA.<sup>11,67</sup> Recently, Markovitsi implied the existence of  
575 “collective” excited states in UVA photon absorption by DNA and speculated that  
576 condensed DNA enhanced the collective character of electronic excitations.<sup>69</sup> The  
577 CTEAB lipoplex made with the plasmid DNA pBR322 raises significantly the SSB  
578 ratio. The effect of the compaction of DNA induced by CTEAB could explain the  
579 increase of the DNA cleavage in the presence of surfactant. Indeed, Merindol *and al.*  
580 have shown the appearance of an absorption band on UVA wavelengths when DNA  
581 was aggregated by temperature.<sup>70</sup> To confirm this hypothesis, UV spectrum of the  
582 CTEAB-DNA complex was performed (see *Supporting Information*): a UVA  
583 absorption band appears in the presence of CTEAB. However, as the solution is  
584 turbid, the light scattering produced by the colloidal dispersion of the lipoplexes  
585 contributes to the UV-Vis spectrum and does not allow to differentiate the  
586 absorbance of the lipoplex from the scattering and to quantify each contribution.

587  
588         The effect of the CTEAB concentration on the DNA photodamage was also  
589 studied and the irradiation time was fixed to 10 min. Indeed, this time allows to see  
590 well the formation of SSB while avoiding overbreaks. The CTEAB concentration  
591 range was from 0 to  $7 \times 10^{-4}$  mol·L<sup>-1</sup> and corresponds to the lanes 2\* to 9\* on the

592 electrophoresis agarose gel shown in figure 11A. In figure 11B, the enhancement of  
 593 the SSB ratio is shown with the increase of the CTEAB concentration.



594

595 **Figure 11. DNA cleavage for different CTEAB concentrations for 10 min**  
 596 **irradiation at  $\lambda \geq 335$  nm and 20°C (\* indicates the irradiated samples). A:**  
 597 **Electrophoresis agarose gel of plasmid pBR322 ( $9 \times 10^{-5}$  mol·L<sup>-1</sup>) in 20 mM**  
 598 **TRIS/HCl buffer (pH = 7.4). Lane 1: DNA alone without irradiation; lanes 2\* to**  
 599 **10\*: DNA with CTEAB irradiated, [CTEAB] = 0,  $1 \times 10^{-5}$ ;  $5 \times 10^{-5}$ ;  $1 \times 10^{-4}$ ;  $2 \times 10^{-4}$ ;**  
 600  **$3 \times 10^{-4}$ ;  $5 \times 10^{-4}$ ;  $7 \times 10^{-4}$  mol·L<sup>-1</sup>. B: Single strand break (SSB) ratio for different**  
 601 **CTEAB concentrations.**

602

603 Two distinct behaviors may be highlighted. For [CTEAB]  $\leq 1 \times 10^{-4}$  mol·L<sup>-1</sup>, single-  
 604 strand breaks increase linearly with CTEAB concentration. Higher concentrations

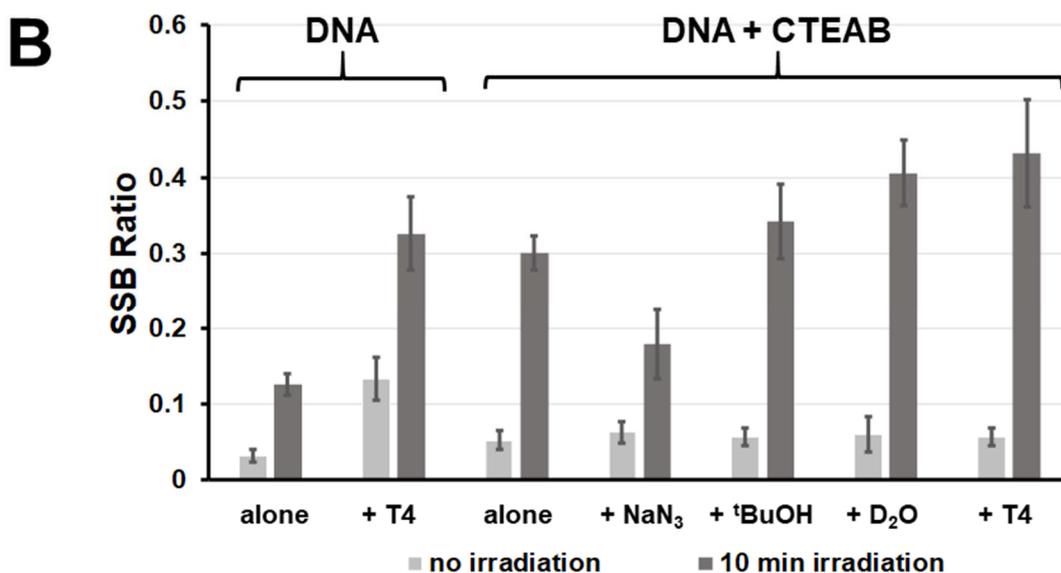
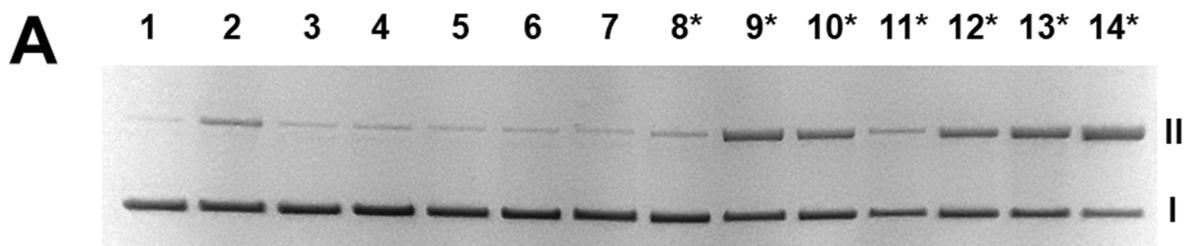
605 give rise to a second compartment which is not linear. The switch between these two  
606 regimes occurs at the characteristic phase transition shown above and corresponding  
607 to the full compaction of DNA by CTEAB.

608 Complementary experiments were undertaken to investigate the mechanism  
609 involved on the DNA damage formed during UVA irradiation of the CTEAB-DNA  
610 lipoplex.

611 One well-known damage induced by UVA irradiation of DNA is the formation of  
612 cyclobutane pyrimidine dimer (CPD)<sup>11,71</sup> and T4 endonuclease V is commonly used  
613 to detect CPD in UV irradiated DNA. Indeed, the presence of CPD on the supercoiled  
614 circular double-strand DNA induced a selective single-strand DNA scission by the  
615 DNA repair enzyme. The plasmid circular form (Form II) appears and can be  
616 revealed by agarose gel electrophoresis and quantified by photodensitometry as  
617 shown in Figure 12. DNA alone was treated with the phage T4 endonuclease V  
618 enzyme before and after 10 min of irradiation at  $\lambda \geq 335$  nm (lanes 2 and 9\*, Figure  
619 12A). The enzymatic treatment reveals, by the AP-lyase activity of the T4  
620 endonuclease V, the presence of abasic lesion on native plasmid DNA without  
621 irradiation (lane 2). When the DNA is irradiated and treated with T4 endonuclease  
622 (lane 9\*), a significantly increase of the single-strand breaks is obtained. This is not  
623 due to the formation of the CPD mediated by UVA radiation, but to the photooxidized  
624 ones. Indeed, the SSB ratio with enzymatic treatment corresponds to the addition of  
625 the native damage in DNA (lane 2) and the photooxidized damage of irradiated DNA  
626 alone (lane 8\*).

627 For the CTEAB-DNA lipoplex sample without irradiation and after T4 treatment (lane  
628 7), the SSB ratio obtained is lower than DNA alone in the same condition (lane 2).  
629 The abasic lesions present in native DNA seem to be no revealed by the T4 enzyme

630 in presence of CTEAB surfactant. It could be due to the quenching of the T4 enzyme  
 631 activity by CTEAB itself or that the enzyme doesn't have access to the lesions  
 632 present on the non-irradiated compacted CTEAB-DNA lipoplex. But when the  
 633 CTEAB-DNA lipoplex is irradiated and treated with the T4 endonuclease (lane 14\*),  
 634 single strand breaks are obtained. As for DNA alone, the observed damage  
 635 enhancement corresponds to the accumulation of the photooxidized damage due to  
 636 the CTEAB-DNA irradiation (lane 10\*) and the abasic native DNA lesions (lane 2). On  
 637 the irradiated sample, the T4 enzyme seems to have access again to the DNA  
 638 damage. Our hypothesis is that the enzyme doesn't have access to the DNA  
 639 because of its compaction in the non-irradiated CTEAB-DNA complex and that the  
 640 photodegradation of the CTEAB-DNA complex induces a partial decompaction,  
 641 enough to give back DNA accessible to the T4 enzyme.



642

643 **Figure 12. DNA cleavage for 10 min of irradiation at  $\lambda \geq 335\text{nm}$  and  $20^\circ\text{C}$  with**  
644 **CTEAB ( $5 \times 10^{-4} \text{ mol}\cdot\text{L}^{-1}$ ) and  $\text{NaN}_3$  ( $200 \times 10^{-3} \text{ mol}\cdot\text{L}^{-1}$ ),  $^t\text{BuOH}$  (4% v/v), in  $\text{D}_2\text{O}$  (80%**  
645 **v/v) or phage T4 endonuclease V treatment (T4) (\* indicates the irradiated**  
646 **samples). A: Electrophoresis agarose gel of plasmid pBR322 ( $9 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$ ) in**  
647 **20 mM TRIS/HCl buffer (pH = 7.4). Lanes 1 and 8\*: DNA alone; lanes 2 and 9\*:**  
648 **DNA + T4; lanes 3 and 10\*: DNA + CTEAB; lanes 4 and 11\*: DNA +CTEAB +**  
649  **$\text{NaN}_3$ ; lanes 5 and 12\*: DNA +CTEAB +  $^t\text{BuOH}$ ; lanes 6 and 13\*: DNA +CTEAB in**  
650  **$\text{D}_2\text{O}$ ; lanes 7 and 14\*: DNA +CTEAB + T4. B: Single strand break (SSB) ratio of**  
651 **CTEAB-DNA lipoplex  $\pm \text{NaN}_3$ ,  $^t\text{BuOH}$ ,  $\text{D}_2\text{O}$  or T4 with and without irradiation.**  
652

653 To go further on the study of the mechanism involved in the formation of SSB,  
654 peculiar scavengers have been used. CTEAB-DNA lipoplex was first irradiated in  
655 presence of sodium azide  $\text{NaN}_3$  (lane 11\*), a well-known singlet oxygen quencher.<sup>72</sup>  
656 Under irradiation, the single-strand break ratio drops in presence of  $\text{NaN}_3$  (Figure  
657 12B). It means that the singlet oxygen is involved on the oxidized damage. To  
658 confirm it, the CTEAB-DNA lipoplex solution was also prepared in  $\text{D}_2\text{O}$  TRIS/DCI  
659 buffer and irradiated for 10 min (lane 13\*). Indeed,  $\text{D}_2\text{O}$  increases the life time of the  
660 singlet oxygen,<sup>73,74</sup> and as expected an enhancement of SSB ratio is obtained (lane  
661 13\*). These results highlight the involvement of a type II mechanism by energy  
662 transfer from the DNA molecule itself, acting as a chromophore,<sup>75</sup> to the ground state  
663 of the molecular oxygen to produce the singlet oxygen responsible of these direct  
664 oxidized photodamage under UVA radiation. Finally, the lipoplex irradiation was  
665 performed with 4 % of tert-butanol ( $^t\text{BuOH}$ , lane 12\*), as a OH radical scavenger.<sup>76,77</sup>  
666 No decrease of the SSB ratio is observable in the presence of  $^t\text{BuOH}$  suggesting the

667 non-intervention of the type I mechanism involving hydroxyl radicals in these  
668 processes.

669 In conclusion, UVA irradiation of DNA-CTEAB lipoplex induces the direct formation of  
670 oxidized damage involving singlet oxygen and implementing energy transfer.  
671 However, it does not lead to the formation of CPD. The compaction of the plasmid  
672 pBR322 by the positive CTEAB surfactant is probably the cause of the increase in  
673 photooxidized DNA damage.

674

675

## 676 **4. Conclusion**

677 Cationic surfactants or polymers have been shown to be efficient complexing agents  
678 and transporters of DNA to cells. In this study, CTEAB surfactant was used to  
679 complex a DNA model, the plasmid pBR322. The self-organization properties of the  
680 CTEAB were studied by different physico-chemical characterizations (tensiometry,  
681 pyrene fluorimetry and scattering experiments). Results show that CTEAB molecules  
682 form small spherical and highly hydrated micelles in TRIS/HCl buffer solution. The  
683 study of the interactions between CTEAB and plasmid DNA has highlighted different  
684 phases, the DNA neutralization, the gradual until the full DNA compaction and the  
685 decompaction process of DNA. Furthermore, formulated CTEAB-DNA lipoplexes  
686 were irradiated under UVA light. With the increase of the irradiation time and of the  
687 CTEAB concentration, an increase of DNA damage was observed. The involvement  
688 of singlet oxygen has been shown to be responsible for these damage. The  
689 compaction of DNA by CTEAB may be the cause of the increase in photooxidized  
690 DNA damage. This study has investigated the DNA compaction by the cationic  
691 surfactant CTEAB and pointed out its role on DNA photodamage under UVA  
692 irradiation. Further work is currently under progress to show what could be the effect  
693 of adding anti-oxidant drug such as vitamin E on the photodamage observed in this  
694 system.

695

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702

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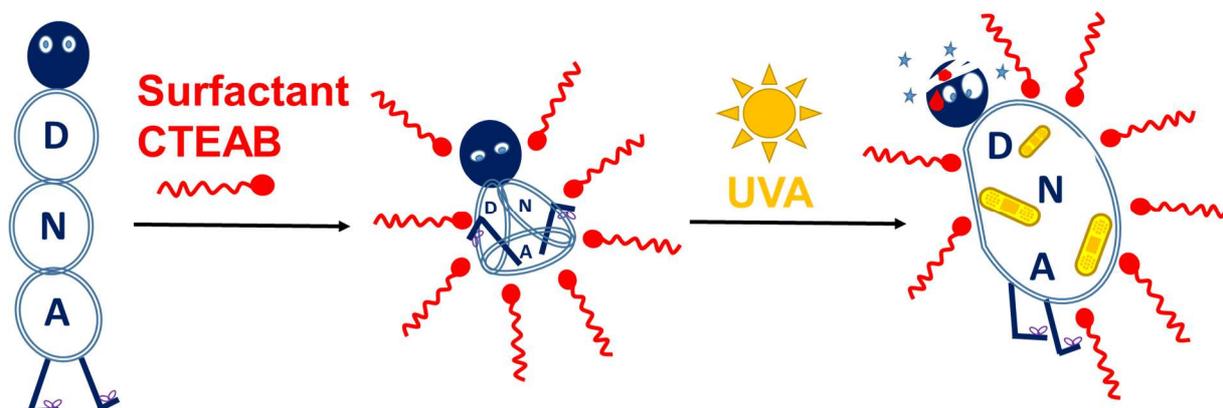
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