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# Aqueous solutions of didecyldimethylammonium chloride and octaethylene glycol monododecyl ether: Toward synergistic formulations against enveloped viruses

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ABSTRACT

Micellization of di-*n*-decyldimethylammonium chloride, **[DiC$_{10}$][Cl]**, and octaethylene glycol monododecyl ether, **C$_{12}$E$_8$**, mixtures have been investigated by surface tension and conductivity measurements. From these results, various physicochemical and thermodynamic key parameters (*e.g.* micellar mole fraction of **[DiC$_{10}$][Cl]**, interaction parameter, free energy of micellization, *etc.*) have been evaluated and discussed in detail. The results prove high synergistic effect between the two surfactants. Based on these results, the virucidal activity of an equimolar mixture of **[DiC$_{10}$][Cl]** and **C$_{12}$E$_8$** has been investigated. A marked synergism was observed on lipid-containing deoxyribonucleic and ribonucleic acid viruses, such as herpes virus, respiratory syncytial virus, and vaccinia viruses. In contrast, Coxsackievirus (non-enveloped virus) was not inactivated. These results support that the mechanism is based on the extraction of lipids and/or proteins from the envelope inside the mixed micelles. This extraction creates "holes" the size of which increases with concentration up to a specific value which triggers the virus inactivation. Such a mixture could be used to extend the spectrum of virucidal activity of the amphiphiles virucides commonly employed in numerous disinfectant solutions.

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## 1. Introduction

Surfactants are one of the most common and most important classes of chemicals widely used in numerous industries (*e.g.* petroleum, cosmetics, foods, pharmaceutics, textiles, *etc.*, Banat et al., 2000). They spontaneously adsorb at liquid/liquid, liquid/solid, liquid/gas and solid/gas interfaces due to their amphiphilic nature leading to reduction of surface and interfacial tensions. Depending on their polar headgroup, they are classified as anionic, cationic, zwitterionic and nonionic (Gambogi et al., 2009). Anionic surfactants are commonly present in cleaning formulations mainly for their detergent and foaming properties (Myers, 2006) while cationic surfactants readily adsorb on negatively charged surfaces making them particularly effective as disinfectants, antistatic agents and fabric softeners. Polyethoxylated nonionic surfactants are primarily used as emulsifiers in various domains such as in cosmetics. They are also often associated with anionic surfactants in detergent formulations because of their efficiency to remove oily soils and because they improve the wettability of the surface by the detergent formulation. They are widely used in many other fields because of their low toxicity and low allergenicity (van Os, 1998). Despite their good hard surface cleaning properties, nonionic surfactants are unfortunately considered as microbiologically inactive (Moore and Payne, 2004). However, some ethoxylated alcohols or related structures have been shown to exhibit a virucidal activity. Although, "virucide" (or "virucidal") is etymologically incorrect since a virus cannot be considered to be living due to the absence of internal metabolism, we have used this term to refer to formulations which are capable to inactivate viruses as often reported in the literature (Steinmann, 2001). The virucidal activity of nonionic surfactants is generally related to their ability to dissolve the viral envelope. For instance, numerous surfactants (CHAPS, Triton X-100, Nonoxynol-9 and Brij-97) are able to solubilize the viral envelope of Sendai, Epstein-Barr and herpes simplex viruses (Ran et al., 1988; Qualtiere and Pearson, 1979; Asculai et al., 1978). However, performing physicochemical or virucidal investigations on such complex systems is far from being straightforward because of (1) the mixtures of several chemical structures (*e.g.* Triton X-100 contains both *para* and *ortho* derivatives in addition to the polydispersity in length of the polyoxoethylene headgroup) and (2) the presence of impurities (e.g. ethylene glycol, transition metal, *etc.*) remaining from the

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synthetic processes. Nevertheless, the use of narrow range and commercially available polyoxyethylene alkyl ethers ($\mathbf{C_iE_j}$) can solve this issue. Among them, the octaethylene glycol monododecyl ether $\mathbf{C_{12}E_8}$ has been shown to solubilize the viral membrane of intact influenza virus (Stegmann et al., 1987). This property has been used to form the so-called virosomes which are unilamellar phospholipid membrane vesicles incorporating virus proteins derived from nucleocapsids. As a consequence, virosomes are able to fuse with cell target and are used for drug or vaccine delivery. The mechanism of solubilization of the viral membrane is based on the accumulation of $\mathbf{C_{12}E_8}$ molecules outside the membrane until the formation of micelles leading to the extraction of membrane constituents (Moore et al., 2006). Therefore, $\mathbf{C_{12}E_8}$ molecules are good candidates to inactivate viruses. As the key parameter to obtain virucidal properties is directly correlated to the $\mathbf{C_{12}E_8}$ micellization, the control of the critical micelle concentration (CMC) can be used to regulate their virucidal properties. This can be achieved through surfactant mixtures (Sugihara et al., 2008). Indeed, nonionic and ionic surfactants mixtures often exhibit functionalities which offer substantial advantages over systems composed of each type of surfactants. Unlike anionic surfactants, one advantage of cationic surfactants is that they are compatible with nonionic ones. Moreover, their mixture often results in a nonideal behavior and the CMC values often decrease (Shiloach and Blankschtein, 1998). This can be accounted for by a reduction of the electrostatic repulsion between the charged surfactants heads favoring the micellization process through addition of the nonionic surfactant. An optimal mixing ratio exists at which the charge screening is maximal leading to formation of mixed micelles at a lower CMC than the sum of the CMC of the individual surfactants (Rauwel et al., 2012).

One of the most widely used cationic surfactants with intrinsic virucidal activity is the di-*n*-decyldimethylammonium chloride, abbreviated as **[DiC$_{10}$][Cl]** (Leclercq et al., 2010a,b, 2012). The mechanism of action is based on the insertion of **[DiC$_{10}$]** cation within the lipid bilayers leading to local variation of the distribution of cationic surfactants between the outer and the inner membrane depending mainly on electrostatic interactions between cationic surfactants and phospholipids (Denyer, 1995). This variation results in morphological changes of the cell or viral membrane that enhance the fluidity leading to the membrane disruption and the cell lysis or virus inactivation (Walsh et al., 2003). In order to comply with the regulation in both the United States of America and the European Union (Nardello-Rataj and Leclercq, 2014) and as the use of these two biocides are authorized, we have explored the synergistic effects between **[DiC$_{10}$][Cl]** and $\mathbf{C_{12}E_8}$ in terms of both micellization and virucidal activity against lipid-containing deoxyribonucleic and ribonucleic acid viruses, such as herpes virus, respiratory syncytial virus, and vaccinia viruses.

## 2. Materials and methods

### 2.1. General information

Well-defined octaethylene glycol monododecyl ether ($\mathbf{C_{12}E_8}$) was purchased from TCI. Its purity, both in terms of alkyl chain length and ethylene oxide distribution was higher than 99.9%. The other reagents were purchased from Sigma-Aldrich Chemical at the highest purity available and were used without further purification. Pure di-*n*-alkyl-dimethylammonium chloride (**[DiC$_{10}$][Cl]**) was synthesized according to the procedure described in our previous work (Leclercq et al., 2010a,b). Ultrapure water was used in all experiments (Millipore water, $\sigma = 72.0$ mN/m, $\kappa = 0.17$ μS/cm at 25 °C). Each surfactant or mixture solutions were prepared extemporaneously.

### 2.2. Surface tension

Surface tensions were measured with the tensiometer K11 (Krüss) using the Wilhelmy plate method. A concentrated solution at a given **[DiC$_{10}$][Cl]** mole fraction ($\alpha_1$) was prepared and the addition of small volumes to ultrapure water ($\sigma = 72.0$ mN/m at 25 °C) was used to increase the solution concentration. After each addition, the solution was gently stirred for 60 s. Surface tension was recorded after equilibration for each mixture. All equilibrium surface tension values were mean quantities of at least three measurements. The precision of the force transducer of the surface tension apparatus was 0.1 mN m$^{-1}$ and before each experiment, the platinum plate was cleaned in red/orange color flame. The temperature was stabilized at 25 ± 0.05 °C with a thermo-regulated bath Lauda RC6. The standard deviation was estimated at ± 10% of the CMC values.

### 2.3. Conductivity measurements

Conductivity measurements were taken with a CDM210 conductivity meter (Radiometer). All measurements were taken in a thermostated water bath. The temperature was stabilized at 25 ± 0.05 °C with a thermo-regulated bath Lauda RC6. A concentrated solution at a given **[DiC$_{10}$][Cl]** mole fraction ($\alpha_1$) was prepared and the addition of small volumes to ultrapure water ($\kappa = 0.17$ μS/cm at 25 °C) was used to increase the solution concentration. After each addition, the solution was gently stirred for 60 s. The conductivity was measured for each surfactant concentration after a sufficient time for the attainment of constant temperature. All conductivity values were mean quantities of at least three measurements. The maximum error limit of conductivity measurements was ± 0.5%.

### 2.4. Dynamic light scattering (DLS)

DLS measurements were performed on a 3D LS Spectrometer (LS instruments, Switzerland) at 25 °C (thermoregulated bath ±0.1 °C). 3D cross correlation mode is used with two APD to improve the detection of small size micelle (<5 nm). 13 angles from 30 to 105° were record to determine the diffusion coefficient ($R_h$ ± 0.1 nm). Cumulant method was apply as data treatment of the correlogram for each angle and polydispersity index was in all case lower than 0.2 that indicate that only monodisperse micelles was observed.

### 2.5. Viruses and cells

HSV-1 (Strain Kos) and VACV (Strain Elstree) were propagated in Vero cells (ATCC® CCL-81™) in Minimum essential Medium (Gibco, Life Technologies) supplemented with 2 mM L-glutamine (Gibco, Life Technologies), 1% non-essential amino-acids (Gibco, Life Technologies) and 2% inactivated fetal calf serum (Gibco, Life Technologies). Cell-free viral suspensions of the viruses were obtained by freezing-thawing cycles followed by a low speed centrifugation to remove cell debris. The very resistant non-enveloped CVB4 (Strain JVB) were propagated in BGM cells in the same medium. RSV (local laboratory strain) was propagated in Hep-2 cells (ATCC® CCL-23™) in the same medium. Viruses titers were assayed by the cytopathic effect of serial dilutions (1:10) of virus-containing samples on Vero, Hep-2 or BGM cells. A sample (100 μL) for each dilution was used to infect four replicate wells in 96-well microtiter plates (Nunclon™ Delta Surface, Thermo Scientific™ Nunc™). Virus-induced cytopathic effects were scored after 5 days of incubation at 37 °C ± 0.1 °C in a humidified 5% $CO_2$ atmosphere. Titers were expressed as the quantity of viruses infecting 50% of the tissue culture wells (Tissue Culture Infectious

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