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How to improve the chemical disinfection of contaminated surfaces by viruses, bacteria and fungus?

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**ABSTRACT**

In response to the current pandemic situation, we present the development of an effective virucidal and biocidal solution to prevent from the spread of infectious diseases through contact with contaminated surfaces. The disinfectants, based on equimolar mixtures of didecyldimethylammonium chloride ([DiC10][Cl]), dodecyloctaglycol (C12E8), and cyclodextrin (CD), show synergistic effects against enveloped viruses (RSV, HSV-1, VACV) and fungi (C. albicans), and additive responses against bacteria (P. aeruginosa). These synergistic mixtures could then be highly helpful for prevention of respiratory illnesses, since a boosted activity allows: (i) a faster eradication of pathogens, (ii) a shorter contact time, and (iii) a complete and broad-spectrum eradication to avoid spread of resistant strains (including bacteria and fungi).

1. Introduction

In the last two decades, three highly pathogenic human coronaviruses (CoV) have emerged. Indeed, after Severe Acute and Middle East respiratory syndromes-related coronaviruses (SARS-CoV-1 and MERS-CoV; Eggers et al., 2015), a novel CoV (named SARS-CoV-2) has been reported from Wuhan, China, in December 2019 (Zhang and Liu, 2020). The World Health Organization has named the CoViD-19 outbreak associated with transmission of this novel CoV. Based on current knowledge, CoViD-19 is spread from person-to-person after close contacts (within about 6 feet) or through respiratory droplets produced when an infected person coughs, sneezes or talks (To et al., 2020; Peng et al., 2020; Lu et al., 2020). Although the transmission occurs much more commonly through respiratory droplets, the persistence of viable SARS-CoV-2 on inanimate dry surfaces for hours to days allows the transmission by self-inoculation (Kampf et al., 2020). Consequently, the spread of CoViD-19 accelerates across continents and a public health crisis as well as a potent economic threat have been observed around the world (Whitworth, 2020).

SARS-CoV-2 is an enveloped virus, meaning that the viral capsid is surrounded by a lipoprotein outer layer (Perlman and Netland, 2009). These viruses are more likely to destruction with a number of physical (e.g. heat, ultraviolet light, etc.) and chemical agents (e.g. bleach, detergents, peroxides, quaternary ammonium compounds, etc.) than non-enveloped viruses (Geller et al., 2012). Therefore, to limit the survival of viruses, disinfection and cleaning of surfaces must be systematically implemented as a prevention measure of CoViD-19 (Dexter et al., 2020). The disinfecting process requires the use of chemical biocides and virucides to inactivate pathogens on surfaces whereas the cleaning step is only used to remove pathogens, dirt and impurities. Both processes lower the number and the risk of transmission.

In this context, the didecyldimethylammonium chloride ([DiC10][Cl]), one of the most widely used quaternary ammonium compounds in healthcare systems to prevent and control viral infections due to its ability to disorganize and to disrupt the envelopes of viruses (Leclercq et al., 2010), is associated with detergents, such as dodecyloctaglycol (C12E8), making the detergent-disinfectant combination ideal for general sanitation purposes (Rauwel et al., 2012). As C12E8 is able to solubilize the viral envelope via micellization, the C12E8 potentiates the virucidal activity of [DiC10][Cl] due to the formation of synergistic co-micelles (Nardello-Rataj and Leclercq, 2016). However, this solution only works with the enveloped viruses since they are more sensitive to destruction than bacteria, fungi and viruses without lipoprotein envelopes (Prince and Prince, 2001). On the other hand, cyclodextrins (CDs) can be used as virucidal agents due to their interactions with the viral-lipids (Carrouel, 2020; Leclercq, 2016). For example, methyl-β-CD alone reduces the infectivity of CoV (Pratelli and Colao, 2015) whereas native γ-CD boosts the virucidal action of [DiC10][Cl] against enveloped viruses since both of them act on the viral-lipids (Leclercq et al., 2016).

It may be hypothesized that [DiC10][Cl]/C12E8/CD systems would demonstrate synergistic effects against enveloped viruses as these
compounds act on the same target (Fig. 1). In order to safe test the virucidal activities, respiratory syncytial virus (RSV), herpes simplex virus type 1 (HSV-1), vaccinia virus (VACV) and coxsackievirus B4 (CVB4), were used instead of hazardous pathogens, including SARS-CoV-2. These synergistic mixtures could then be highly helpful for prevention of viral respiratory illnesses, since a boosted activity allows: (i) a faster eradication of viruses, (ii) a shorter contact time, and (iii) a complete and broad-spectrum eradication to avoid spread of resistant pathogens (e.g. bacteria and fungi).

2. Materials and methods

2.1. General information

Didecyldimethylammonium chloride ([DiC10]Cl) was as synthesized according to the procedure described in our previous work (Leclercq et al., 2010). Dodecyl octaglycol (C12E8) was purchased from Sigma-Aldrich Chemical at the highest purity available. The other chemical compounds were purchased as mentioned below. Sterile water was used in all experiments. Each solution was prepared extemporaneously. The composition of the neutralizer and diluent was: phosphatidylcholine, 3 g; Tween 80, 1 g; saponin, 30 g; tryptone salt, 9.5 g; water qs. 1 L. Experiments were performed at 25 ± 0.1°C.

2.2. Propagation of the test viruses

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), 1% non-essential amino-acids (Gibco) and 2% inactivated fetal calf serum (Gibco). The 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. Cell-free viral suspensions of the viruses were obtained by freezing-thawing cycles followed by a low speed centrifugation to remove cell debris. Viruses titers were assayed by the cytopathic effect of serial dilutions (1:10) of virus-containing samples on Vero or Hep-2 cells. A sample (100 μL) for each dilution was used to infect four replicate wells in 96-well microtitre plates (Nunc™ Delta Surface, Thermo Scientific™ Nunc®). The solutions were placed in light-scattering cells (10 mm) to determine the hydrodynamic diameter (Dh) using 3D LS Spectrometer (LS instruments) at 25 ± 0.05°C. 3D cross correlation mode was used with two APD to improve the detection of small size micelle (< 5 nm) at 90°. CMC were prepared and the critical micelle concentrations (CMCs) were determined experimentally and used to calculate the CMC (± 10% of the value).

2.3. Virucidal assay

200 μL of the viral stock solution was added to 200 μL of surfactant (C12E8 and/or [DiC10]Cl) or 200 μL of the aqueous ternary mixtures of CD, C12E8 and [DiC10]Cl (all solutions were in equimolar proportions). After incubation for 15 min (or 2 h) at 25 ± 0.1°C, the mixtures were immediately filtered on MicroSpin S-400 HR columns (GE Healthcare) to separate viruses from the other components of the mixtures. Residual viruses were then titrated as described above. Each experiment was performed at least twice. The virucidal activity was determined by the difference of the logarithmic titer of the virus control minus the logarithmic titer of the test virus. This difference is presented as a reduction factor including its 95% confidence interval. A reduction in virus titer of ≥4-log10 was regarded as evidence of sufficient virucidal activity according to EN 14476. The lowest concentration giving a reduction in virus titer of ≥4-log10 was the minimum virucidal concentration (MVC).

2.4. Critical micelle concentration

Various solutions of [DiC10]Cl, C12E8 and CD alone or in mixture were prepared and the critical micelle concentrations (CMCs) were estimated by surface tension measurements using the Wilhelmy plate method (Tensiometer K100, Krüss) at 25 ± 0.05°C. 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 measurement, the platinum plate was cleaned in red/orange color flame. Variations of air/water surface tension as a function of total surfactant concentration were plotted. As the surface tension is linearly correlated to the surfactant concentration in logarithmic scale in both pre- and post-micellization regions, the intersection point of these two straight lines corresponds to the CMC (± 10% of the value).

2.5. Dynamic light scattering (DLS)

The solutions were placed in light-scattering cells (10 mm) to determine the hydrodynamic diameter (Dh) using 3D LS Spectrometer (LS instruments) at 25 ± 0.05°C. 3D cross correlation mode was used with two APD to improve the detection of small size micelle (< 5 nm) at 90°. Cumulant method was applied as data treatment of the correlogram and polydispersity index was in all case lower than 0.2. The analysis provides an average diffusion coefficient used to calculate a Dh ± 0.2 nm using the Stokes-Einstein equation.

2.6. ζ-potential

The measurements were performed using a Zetasizer Nano ZS (Malvern Instruments) at 25 ± 0.05°C. Three runs per sample were used to establish measurement repeatability. The applied voltage can be set to automatic mode (start at a low voltage, typically 80 V, and increase the voltage gradually to 150 V). The electrophoretic mobility was determined experimentally and used to calculate the ζ-potential.

2.7. Apparent degree of micelle ionization

The measurements were taken with a CDM210 conductivity meter (Radiometer). All measurements were taken at 25 ± 0.05°C. As the conductivity is linearly correlated to the surfactant concentration in both pre- and post-aggregation regions, the ratio between the two slopes gives the apparent degree of micelle ionization (± 3%).
2.8. Bactericidal assay

All bactericidal tests were performed using *Pseudomonas aeruginosa* (ATCC® 15442™). They were carried out in accordance with European standard EN 1040. A sample of the product was diluted with water and a test suspension of bacteria cells was added. The number of bacteria cells in the suspension was adjusted between 1.5 × 10^7 and 5.0 × 10^7 CFU/mL. The mixture was maintained at 20 ± 1°C for 5 min ± 10 s. At the end of this contact time, an aliquot was taken; the bactericidal activity in this portion was immediately neutralized. The number of surviving yeasts in each sample was determined. Samples were serially diluted from 10^-1 to 10^-5 and each dilution was plated in duplicate on tryptone soya agar. After 48 h incubation of the plates at 37°C, the colony-forming units per millimeter (CFU/mL) were counted. The bactericidal activity was determined by the difference between the logarithmic number of CFU/mL in the suspension at the beginning of the contact time (control test) and the logarithmic number of survivors per mL. This difference is presented as a reduction factor including its 95% confidence interval. A reduction of ≥4-log_{10} (corresponding to a destruction of ≥99.99%) was regarded as evidence of sufficient bactericidal activity. The lowest tested concentration giving a reduction factor of at least 4 compared to the control was defined as the minimum bactericidal concentration (MBC).

2.9. Fungicidal assay

All fungicidal tests were performed using *Candida albicans* (ATCC® 10231™). They were carried out in accordance with European standard EN 1275. A sample of the product was diluted with water and a test suspension of yeast cells was added. The number of yeast cells in the suspension was adjusted between 1.5 and 5.0 × 10^6 CFU/mL. The mixture was maintained at 20 ± 1°C for 15 min ± 10 s. At the end of this contact time, an aliquot was taken; the fungicidal activity in this portion was immediately neutralized. The number of surviving yeasts in each sample was determined. Samples were serially diluted from 10^-1 to 10^-6 and each dilution was plated in duplicate on Sabouraud dextrose agar. After 48 h incubation of the plates at 37°C, the colony-forming units per millimeter (CFU/mL) were counted. The fungicidal activity was determined by the difference between the logarithmic number of CFU/mL in the suspension at the beginning of the contact time (control test) and the logarithmic number of survivors per mL. This difference is presented as a reduction factor including its 95% confidence interval. A reduction factor of ≥4 was regarded as evidence of sufficient fungicidal activity according to EN 1275. The lowest concentration giving a reduction in virus titer of ≥4-log_{10} was the minimum fungicidal concentration (MFC).

3. Results and discussion

3.1. Virucidal performance and mechanism against lipid-containing RNA viruses

Virucides, that attack and inactivate viral particles outside the cell (virions) by damaging their envelopes (and/or capsids) or genomes, are widely used to disinfect hard surfaces. This practice is useful for the prevention viral respiratory illnesses (e.g. CoVID-19, RSV, etc.) in households and community settings. Indeed, RSV is an enveloped RNA virus that primarily infects human epithelial cells within the nasopharynx. Infection with RSV is generally exhibited as lower respiratory tract disease, pneumonia, bronchiolitis or tracheobronchitis (Tang and Crowe, 2007). Although all individuals can be infected, those at high risk include premature infants, young children, elderly, immunocompromised, and children under age 2 with chronic lung conditions. For these patients, RSV can cause pneumonia, leading to severe respiratory illness requiring hospitalization and causing sometimes death (Shi et al., 2020). For instance, within USA, 100,000 hospitalizations and 4,500 deaths annually are attributed to RSV infections (Tang and Crowe, 2007). The treatment of RSV disease is essentially limited to supportive care such as oxygen therapy. Indeed, to treat severe RSV infections ribavirin is used but recent studies suggest that its use produces no benefit (Tang and Crowe, 2007). It is noteworthy that RSV is also a major cause of nosocomial infections. If infection among healthy and immunocompetent individuals tends to be less severe, some specific factors may predispose to RSV infection include: crowding, exposure to tobacco and smoke, low socioeconomic status, and family history of atopy and asthma. Note that these factors are similar to those of SARS-CoV-2 infections (Zhang and Liu, 2020). In contrast, RSV is more vulnerable to SARS-CoV-2 to environmental changes, particularly temperature and humidity: it loses up to 90% infectivity at room temperature after 48 hours (Tang and Crowe, 2007). However, it may survive 3 to 30 hours on nonporous surfaces at room temperature (Tang and Crowe, 2007). To prevent RSV infections, disinfectants based on sodium hypochlorite (1%), formaldehyde (18.5 g/L), glutaraldehyde (2%), iodine (1%), sodium deoxycholate (0.1%), sodium dodecyl sulphate and Triton X-100 can be used (Tang and Crowe, 2007; World Health Organization, 1993). On the other hand, human CoV are susceptible to sodium hypochlorite (0.1%), organochlorine (0.1%), iodine (10%), ethanol (70%) and glutaraldehyde (2%) but more or less resistant to quaternary ammonium compounds according to their structures (Sattar et al., 1989). For instance, [DiC10] [Cl] was able to reduce the viral loading of canine CoV by > 4.0 log_{10} but benzonium chloride only by 3.0 log_{10} despite the fact that both agents caused significant morphological damage to the virus (Pratelli, 2007). Therefore, Schrank et al. (2020) claimed that: “this pandemic serves as an opportunity for enhanced antiseptics, and more specifically quaternary ammonium compounds development, as commercially available disinfectants have room for improvement both with formulation and concentration as well as effectiveness against both viral and bacterial contagions”. As, some ethoxylated surfactants (e.g. Triton X-100, Nonoxynol-9 or Brij-97) have been shown to exhibit a virucidal activity due to their ability to solubilize the viral envelope of Epstein-Barr or herpes simplex viruses (Qualtiere and Pearson, 1979; Ascui et al., 1978), and, as, Cds “are able to participate in the attack of viruses, and specifically SARS-CoV-2, in a large range of different ways” (Garrido et al., 2020), we have investigated [DiC10] [Cl]/C12E8/CD ternary systems to obtain synergistic effects against enveloped viruses such as RSV instead of hazardous SARS-CoV-2.

In this context, we first determined the virucidal activity of [DiC10] [Cl] (Q), C12E8 (E), and CD (a, β or γ) mixtures against RSV (Table 1). Secondly, some physicochemical properties of these mixtures were recorded to establish the mechanism of action of these mixtures (Table 2). As control experiments, we evaluated the virucidal activity of each compound alone or in binary mixture against RSV at various concentrations. It is noteworthy that a reduction in virus titer of ≥4-log_{10} (corresponding to an inactivation of ≥99.99%) was required to claim a virucidal, disinfectant and antiseptic efficacy according to EN 14476. Therefore, the lowest concentration able to inactivate at least 99.99% of viruses is taken as the minimum virucidal concentration (MVC) of the compounds. Thus, the MVC were estimated to 125 µM for [DiC10] [Cl] and C12E8 after 15 min of contact time. For [DiC10] [Cl], MVC is lower than the CMC (1,200 µM; see Table 2). As previously reported this observation supports the insertion of free [DiC10] cation within the viral envelope leading to membrane disorganization until destruction and virus inactivation (Leclercq et al., 2016). The opposite holds for C12E8; the MVC value is higher than the CMC of the surfactant (125 vs. 100 µM; see Table 2) which suggests that the C12E8 micelles are the active species (Nardello-Rataj and Leclercq, 2016). It is noteworthy that the hydrodynamic diameter (Dh) of the micelle was estimated to be around ~8 nm while the diameter of intact RSV was found at ~200 nm (see Table 2; Utley et al., 2008). The adsorption of micelles on the viral envelope leads to the C12E8 insertion in the outer membrane associated with a rapid flip-flop across the lipid membrane leading to the...
It is noteworthy that for CD, the (CD)/MVC is very high and [CD] very weak (see above and Table 1). A value of SI < 1 indicates a synergistic effect while a value SI > 1 means an antagonist effect (Zwart Voorspuij and Nass, 1957). Finally, when SI = 0, simple additivity is observed between the components. As presented in Tables 1 and 2, for all the [DiC10][Cl]/CD mixtures, the MVCs are lower than the CMCs suggesting that the micelles are inactive species. Furthermore, as the CD affinity for solubilizing lipids are in the order γ-CD < β-CD < α-CD for phospholipids and α-CD < γ-CD < β-CD for cholesterol (Ohtani et al., 1989), we can reasonably suppose that all [DiC10][Cl]/CD mixtures must provide either additive or synergistic response due to concomitant effects of both compounds on the viral envelope (see above) and that the efficiency is linked to the affinity of CDs for viral-lipids. Unfortunately, only the [DiC10][Cl]/γ-CD mixture is synergic (SI = 0.6, Table 1). The two other [DiC10][Cl]/CD combinations result in antagonistic responses. Therefore, the virucidal efficiency is also linked to the [DiC10][Cl]/CD binding constants that increases in the order γ-CD < β-CD < α-CD (Table 2, Funasaki et al., 2000). As a given free [DiC10] concentration is required to sufficiently disorganize the viral envelope and to allow the lipid extraction mediated by CDs, the formation of stable complexes between the [DiC10] cation and α- or β-CD tends to limit the membrane disorganization-induced by the cation whereas this behavior is less strong with γ-CD due to the weak binding constant. Therefore, the efficiency of [DiC10][Cl]/CD mixtures is more linked to the equilibrium constants, rather than the affinity of CDs for lipids. For C12E8/CD mixtures, the responses are only antagonist (with α-CD and β-CD) or additive (for γ-CD; see SI values, Table 1). As previously mentioned, CDs inhibit the micellization as indicated by the observed increase in the CMC with CDs. The degree of micelle inhibition (linked to the affinity of CDs for viral-lipids) is linked to the affinity of CDs for viral-lipids. Unfortunately, only the [DiC10][Cl]/CD mixtures must provide either additive or synergistic effect due to concomitant effects of both compounds on the viral envelope (see above) and that the efficiency is linked to the affinity of CDs for viral-lipids. Unfortunately, only the [DiC10][Cl]/γ-CD mixture is synergic (SI = 0.6, Table 1). The two other [DiC10][Cl]/CD combinations result in antagonistic responses. Therefore, the virucidal efficiency is also linked to the [DiC10][Cl]/CD binding constants that increases in the order γ-CD < β-CD < α-CD (Table 2, Funasaki et al., 2000). As a given free [DiC10] concentration is required to sufficiently disorganize the viral envelope and to allow the lipid extraction mediated by CDs, the formation of stable complexes between the [DiC10] cation and α- or β-CD tends to limit the membrane disorganization-induced by the cation whereas this behavior is less strong with γ-CD due to the weak binding constant. Therefore, the efficiency of [DiC10][Cl]/CD mixtures is more linked to the equilibrium constants, rather than the affinity of CDs for lipids. For C12E8/CD mixtures, the responses are only antagonist (with α-CD and β-CD) or additive (for γ-CD; see SI values, Table 1). As previously mentioned, CDs inhibit the micellization as indicated by the observed increase in the CMC with CDs. The degree of micelle inhibition (linked to the C12E8/CD binding constants, Table 2) is in order γ-CD < α-CD < β-CD which is in perfect agreement with the virucidal efficiency (β-CD < α-CD < γ-CD, compare Tables 1 and 2). This observation supports the mechanism based on the micellar extraction of lipids from the viral envelope.

Before considering the ternary mixtures, a third series of control experiments was performed in the presence of [DiC10][Cl] and C12E8. The CMC of mixed surfactant (60 µM) was sharply lower than that of sole surfactants (1,200 and 100 µM for [DiC10][Cl] and C12E8, see Table 2) due to favorable interactions between the components in the

### Table 1

| Concentration of each component (µM) | SI (α, β) |
|-------------------------------------|----------|
|                                     | (≤1.0)   |
| Q                                   | 1.0 ≤ 2.0, 3.0 ≤ 5.0 ≤ 7.5 ≤ 12 ≤ 25 ≤ 50 ≤ 100 |
| Q/α                                 | 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |
| Q/β                                 | 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |
| Q/γ                                 | 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |
| E                                   | 0.8 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |
| E/α                                 | 0.8 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |
| E/β                                 | 0.8 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |
| E/γ                                 | 0.8 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 ≤ 1.0 |

\[
SI = \frac{[X]}{MVC_X}
\]

where \([X]\) is the concentration of \(X\) component in the mixture that produced a virucidal action (i.e. a reduction in virus titer of ≥4-log 10) and MVC\(_X\) is the MVC observed for \(X\) acting alone (\(X = Q, E, \text{or } CD\)). It is noteworthy that for CD, the (CD)/MVC is very high and [CD] very weak (see above and Table 1). A value of SI < 1 indicates a synergistic effect while a value SI > 1 means an antagonist effect (Zwart Voorspuij and Nass, 1957).

### Table 2

| Micelles properties | Binding constant (M⁻¹) |
|---------------------|-----------------------|
|                     | K\(Q\) | K\(E\) | K\(Q\) | K\(E\) |
| Q                   | 1,200  | 4.0    | 57.9   | 64    |
| Q/α                 | 5,500  | 3.9    | 60.4   | 60    |
| Q/β                 | 5,100  | 4.1    | 54.9   | 63    |
| Q/γ                 | 10,200 | 4.0    | 61.7   | 58    |
| E                   | 100    | 7.9    | 0      | -     |
| E/α                 | 160    | 8.1    | 0      | -     |
| E/β                 | 200    | 7.7    | 0      | -     |
| E/γ                 | 105    | 8.0    | 0      | -     |
| Q/E                 | 60    | 8.5    | 35.4   | 73    |
| Q/E/α               | 90    | 8.7    | 38.7   | 75    |
| Q/E/β               | 80    | 8.6    | 40.1   | 76    |
| Q/E/γ               | 70    | 8.4    | 34.2   | 72    |

\(a\) Virucidal activity in log₁₀ titer reduction factor recorded after 15 min of contact time at room temperature with an initial RSV of 1.3 × 10⁷ TCID₅₀/mL (with α = CD, β = β-CD, γ = γ-CD, Q = [DiC10][Cl] and E = C₁₂E₈).

\(b\) Calculated according equation 1.

\(c\) Minimum virucidal concentration (MVC) = the lowest concentration able to inactivate at least 99.9% of viruses.

\(d\) Taken in Leclercq et al., 2013.

\(\ast\) All binary or ternary systems are equimolar.

\(\ast\) CMC = critical micelle concentration, \(D_θ\) = hydrodynamic diameter, ζ-potential recorded at 10 × CMC, α = the degree of ionization of the micelle.

\(\dagger\) Determined from surface tension modelling using single surfactant/cyclodextrin systems in aqueous solution with dilution experiments.

\(\ddagger\) Taken in Leclercq et al., 2013.
co-micelles. On the other hand, according to the data reported in Table 1, the [DiC10][Cl] and C12E8 mixture is synergistic (SI = 0.6). As the MVC value (100 µM, Table 1) is clearly much higher than the CMC of [DiC10][Cl]/C12E8 mixture (60 µM, Table 2), a mechanism based on the micellar extraction of lipids from the viral envelope is always considered as highly probable. In addition, unlike RSV that presented at least 6.5-log10 reduction at 50 µM of [DiC10][Cl] and 50 µM of C12E8, only 1-log10 reduction was observed for CBV4 (non-enveloped virus) with the [DiC10][Cl]/C12E8 mixtures (1,000 µM of each) in 15 min. Therefore, the virucidal mechanism of mixtures can be described by the disruption or detachment of the viral envelope. The physicochemical data reveal that the sizes of the co-micelles are substantially identical to those of the pure C12E8 micelles but higher than those of the pure [DiC10][Cl] micelles (compare the hydrodynamic diameters, Dho, in Table 2). This observation supports that the co-micellization is governed by the C12E8 molecules. Second, the ζ-potential of co-micelles was weaker than that of the [DiC10][Cl] micelles whereas the degree of co-micelle ionization (α) was higher. These findings are due to the “pandant” effect of the polyoxyethylene chains (non-enveloped virus) as an open-chain equivalent to a crown ether able to complex [DiC10] cations (Rauwel et al., 2012). This cation binding pandant decreases the electrostatic repulsion between the [DiC10] cations, takes the chloride ions away and stabilizes the co-micelles. Based on these observations, we can suppose a relationship between the ζ-potential of the micelles and the virucidal activity. Indeed, the high ζ-potential of co-micelles compared to pure C12E8 micelles improves the probability of the attachment of [DiC10] cations to the anionic regions (i.e. phospholipids and proteins) prior to adsorption inside the envelope (Tobe et al., 2015). It is believed that increasing the appearance of the cationic region of [DiC10] cations in mixed micelles will increase the adsorption of [DiC10] cations on the anionic region of the viruses. This behavior associated with the weak CMC of the mixed system is supposed to be responsible for the observed synergistic effect.

The CMCs of [DiC10][Cl]/C12E8/CD ternary mixtures were clearly lower than that of sole surfactants (1,200 and 100 µM for [DiC10][Cl] and C12E8, see Table 2) due to favorable thermodynamic interactions between the components in the co-micelles. However, this effect is less important in the presence of CDs than without (60 µM, see Table 2). Moreover, the [DiC10][Cl]/C12E8/CD ternary systems are very stable. Indeed, the stability of micelles can be described in terms of thermodynamic and kinetic stability. Kinetic stability describes the behavior of the system over time whereas thermodynamic stability describes how the system acts as micelles are formed and reaches equilibrium. To characterize the thermodynamic stability of micelles, the key parameter is the CMC (Zana, 1996). Indeed, lower values indicate greater thermodynamic stability as the CMC is directly related to the standard free energy of micellization according to the following equation:

\[ \Delta G_{\text{mic}} = RT \ln(\text{CMC}) \]  

(2)

In all cases, the observed CMCs of [DiC10][Cl]/C12E8/CD ternary systems are clearly inferior to those of the surfactants alone (see Table 2). However, these micelles can be disassembled if some components undergo a chemical degradation. For instance, the temperature can be detrimental to the stability of micelles due to a possible Hofmann elimination on the [DiC10][Cl] surfactants. Therefore, the stability of the [DiC10][Cl]/C12E8/CD ternary systems has been investigated at 50°C for one week to accelerate the stability tests. In such conditions, the NMR and FTIR analyses do not detect the presence of olefins or other-side products. In addition, the hydrodynamic diameters and the ζ-potentials also remain unaffected. Thus, the micelles, the surfactants and the CDs provide stable micellar systems at higher temperature. In contrast, a drastic (+ 20%) increase of olefin arisen from the cationic surfactant was observed after 6 hours at pH 10 in the presence of sodium hydroxide at 50°C. Such degradation obviously impacts the stability of the micelles. Fortunately, under classical storage conditions, and in particular in a neutral environment, when no chemical degradation takes place, the micelles formed by the [DiC10][Cl]/C12E8/CD ternary system and the resulting formulations exhibit a very long-term thermodynamic stability. In term of virucidal activity against RSV, the [DiC10][Cl]/C12E8/CD mixture ternary mixtures were synergetic. However, the mixtures that use β- and γ-CD (SI = 0.6) were more synergetic than with α-CD or without CD (SI = 0.8, Table 1). It is noteworthy that the mixtures based on β- and γ-CD were also active than the [DiC10][Cl]/γ-CD binary system (SI = 0.6). Despite the advantage of only using two components instead of three, two serious drawbacks can be pointed out: (1) the [DiC10][Cl]/γ-CD binary mixture is only disinfectant where the [DiC10][Cl]/C12E8/γ-CD is a disinfectant cleaner and (2) the γ-CD is more expensive than the β-CD. As the sizes of the co-micelles as well as the ζ-potentials and the degrees of ionization of micelle (α) were not influenced by the presence of CDs (see Table 2), we can suppose that the CDs as well as the inclusion complexes remain in solution and not in the vicinity of the micelle shell. Therefore, the good virucidal activities may be the result of the combined effect of [DiC10]/C12E8 (envelope disorganization and lipids extraction) and CDs (lipids complexion) leading to the “solubilization” of the viral envelope and to the full leakage of internal constituents. Indeed, as the surface of enveloped viruses is composed of spike proteins and lipids, these are the most likely targets of ternary mixtures as well as of binary ones or each component alone. Based on this assertion and the virucidal mechanisms previously proposed (see below), the potentiation can be ascribed to the following sequential steps: (i) the formation of [DiC10][Cl]/C12E8 co-micelles, (ii) the adsorption at the membrane lipidic interface, (iii) the fast insertion/removal between the [DiC10] cations and the lipids, (iv) the alteration of membrane properties facilitating the lipid extraction by the CDs, and (v) the cumulative damages on the membrane lead to exposure of viral core genome (Fig. 2). It is noteworthy that a concomitantly mechanism in which free [DiC10] cations interact, penetrate and weaken the viral envelope due to the multiple equilibria in [DiC10][Cl]/C12E8/CD ternary systems also take place (see Fig. 2). Indeed, next to the [DiC10][Cl]/C12E8 co-micellization equilibrium, the introduction of C12E8 in [DiC10][Cl]/CD system results in the reduction of the CD’s affinity for [DiC10] due to the C12E8 binding to the CD. This new equilibrium decreases the free CD concentration. Le Chatelier’s principle opposes this decrease and attempts to make up for the loss of CD: more free [DiC10] cations are then obtained. In the presence of viruses, the free [DiC10] cation can be interacted with the viral envelope. Due to the insertion in the envelope, the free [DiC10] cation concentration decreases, altering the equilibria, so more [DiC10] cation and CD are released. At this stage, CDs are able to extract lipid from the viral envelope by complexion thus creating “holes”. Consequently, in this mechanism...
the [DiC10]/C12E8 co-micelles as well as the various [DiC10]/CD inclusion complexes can be seen as reservoirs of [DiC10] and CDs which are readily available for interaction with the viral envelope (i.e. complexation and insertion, for CDs and [DiC10], respectively).

3.2. Virucidal and biocidal activity against DNA enveloped viruses, bacteria and fungus

To extend the scope of the systems, the biocidal (or virucidal) action against DNA enveloped viruses, bacteria and fungus was carried out under similar conditions (Table 3). The two selected lipid-containing DNA viruses are the well-known HSV-1 and VACV. The HSV viruses lead to orofacial, ophthalmic and genital herpes, and sometimes to DNA viruses are the well-known HSV-1 and VACV. The HSV viruses against DNA enveloped viruses, bacteria and fungus was carried out and fungus could lead to orofacial, ophthalmic and genital herpes, and sometimes to DNA viruses are the well-known HSV-1 and VACV. The HSV viruses

| Concentration of each component (µM) | 20 | 35 | 50 | 75 | 125 | 250 | 500 | 1000 |
|-------------------------------------|----|----|----|----|-----|-----|-----|------|
| HSV-1 (†, α, β or γ)                |    |    |    |    |     |     |     |      |
| Q                                  | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 |
| Q/E                               | ≤1.0 | 1.1 | 1.5 | 3.7 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/α                              | ≤1.0 | 2.6 | 2.6 | 3.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/β                              | ≤1.0 | 3.7 | 5.7 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/γ                              | ≤1.0 | 2.2 | 6.0 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/β                              | 1.3 | 4.0 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/γ                              | 4.1 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| VACV (†, α, β or γ)               |    |    |    |    |     |     |     |      |
| Q                                  | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 |
| Q/E                               | ≤1.0 | 1.2 | 2.8 | 4.6 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/α                              | ≤1.0 | 1.3 | 4.0 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/β                              | ≤1.0 | 1.3 | 5.2 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/γ                              | ≤1.0 | 1.0 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| P. aeruginosa (†, α, β or γ)      |    |    |    |    |     |     |     |      |
| Q                                  | 1.7 | 3.2 | 4.1 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| E                                  | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 |
| Q/E                               | 1.7 | 3.6 | 4.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/α                              | 1.4 | 2.7 | 4.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/β                              | ≤1.0 | 2.1 | 4.6 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| Q/E/γ                              | 1.2 | 2.1 | 4.1 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 | ≥6.5 |
| C. albicans (†, α, β or γ)        |    |    |    |    |     |     |     |      |
| Q                                  | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 |
| Q/E                               | ≤1.0 | 1.3 | 4.3 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 |
| Q/E/α                              | ≤1.0 | 1.1 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 | ≤1.0 |
| Q/E/β                              | ≤1.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 |
| Q/E/γ                              | ≤1.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 |
| Q/E/γ                              | ≤1.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 | ≥6.0 |

Table 3: Dose-dependent biocidal or virucidal activity and corresponding synergy index (SI) against enveloped DNA viruses (HSV-1 and VACV), bacterium (P. aeruginosa) and fungus (C. albicans).

### Notes:

- Biocidal or virucidal activity in log10 titer reduction factor recorded at room temperature after 15 min of contact time for HSV-1 (1.5 × 10⁸ TCID₅₀/mL), VACV (1.1 × 10⁵ TCID₅₀/mL), C. albicans (1.6 × 10⁵ CFU/mL) and 5 min for P. aeruginosa (1.0 × 10⁶ CFU/mL) with α = α-CD, β = β-CD, γ = γ-CD, Q = [DiC10][Cl] and E = C₁₂E₈.

- Calculated according equation 1.

- Minimum virucidal concentration (MVC), minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) = the lowest concentration able to reduce the titer of at least 99.99% of viruses, bacteria, or fungus, respectively.

- Table 3: The SI values were comprised between 0.8 and 0.2. However, the extent of synergistic effect for [DiC10][Cl]/C₁₂E₈/CD mixture was in the order HSV-1 (SI = 0.2) = RSV (SI = 0.3) > VACV (SI = 0.6). In addition, the virucidal activities of [DiC10][Cl]/C₁₂E₈/CD mixtures (defined as 1/MVC) were in the following order: HSV-1 > RSV > VACV (Tables 2 and 3). This differential susceptibility is correlated with the virus size: the diameters are ~120, ~200 and ~320 nm for HSV-1, RSV and VACV (Salmon et al., 1998; Utley et al., 2008; Malkin et al., 2003). Indeed, based on the proposed virucidal mechanism (see above), [DiC10]/C₁₂E₈ co-micelles and [DiC10] alone can interact and alter the viral envelope, after the insertion of [DiC10] cations, facilitating the lipid extraction by the CDs and leads to virus inactivation. We can therefore suppose that the required amount of inserted [DiC10] cations to obtain a sufficient disorganization of the membrane prior to virus inactivation is higher for VACV than RSV or HSV-1 because of the viral size.

As expected, the C₁₂E₈ or CDs alone were inactive against P. aeruginosa and C. albicans, but [DiC10][Cl] was active against both pathogens. The [DiC10][Cl]/C₁₂E₈ and [DiC10][Cl]/C₁₂E₈/CD mixtures were also active against P. aeruginosa that [DiC10][Cl] alone. Therefore, only additive responses were obtained for binary and ternary mixtures (SI = 1, Table 3). In contrast, all the [DiC10][Cl]/C₁₂E₈ and [DiC10][Cl]/C₁₂E₈/CD combinations were synergistic against C. albicans. However, the extent of synergistic effect for [DiC10][Cl]/C₁₂E₈/CD mixtures was in the order α-CD (SI = 0.5) = β-CD (SI = 0.5) > γ-CD (SI = 0.7). The presence of CD in the ternary medium did not improve the fungicidal activity of [DiC10][Cl]/C₁₂E₈ binary mixture (SI = 0.5, Table 3). Despite the fact that the synergistic effect was not improved against P. aeruginosa and C. albicans, the bactericidal and fungicidal activities are suitable to obtain broad-spectrum biocidal or virucidal.
formulations due to the synergistic effects observed against enveloped viruses. Indeed, the equimolar \([\text{DiC}10]([\text{Cl}]/\text{C12E8})/\beta-\text{CD}\) mixture (50 µM of each) is able to fight RSV, HSV-1, VAC, \textit{P. aeruginosa}\) and \textit{C. albicans}\) whereas the equimolar \([\text{DiC}6]([\text{Cl}]/\text{C12E8})\) mixture (50 µM of each) is inactive on VAC and \([\text{DiC}0]([\text{Cl})\) alone (50 µM) is only active against \textit{P. aeruginosa}.

3.3. Comparison with commercial products

It should be noted that the vast majority of disinfectant products currently on the market, have at least two active ingredients. For instance, Health Canada reports that for 167 approved disinfectants (solution, spray or gel), only 19 products use the \([\text{DiC}0]([\text{Cl})\) alone and 2 products are up to 6 active ingredients (Health Canada, 2020a). \([\text{DiC}0]([\text{Cl})\) is usually in mixture with other biocidal compounds: quaternary ammonium ([DiC6][Cl], [C6C10][Cl], benzalkonium chloride) and/or alcohols (ethanol, isopropyl alcohol) and/or organic acids (citric acid), and/or essential oils (pine oil). The composition of products with \([\text{DiC}10]([\text{Cl})\) alone ranges from 0.052 wt.% for ready-to-use (KLERICIDE RTU by Lonza LLC; Health Canada, 2020a). For the mixtures, the lowest \([\text{DiC}10]([\text{Cl})\) concentration is found to be 0.0083 wt.% supple

References

Asculi, S.S., Weis, M.T., Rancourt, M.W., Kupferberg, A.B., 1978. Inactivation of herpes simplex viruses by nonionic surfactants. Antimicrob. Agents Chemother. 13, 696–699.

Carrouel, F., Conte, M.P., Fisher, J., Gonçalves, L.S., Dussart, C., Llodra, J.C., Bourgeois, D., 2020. COVID-19: A recommendation to examine the effect of mouthrinses with β-cyclodextrin combined with citrox in preventing infection and progression. J. Clin. Med. 9, 1126. https://doi.org/10.3390/jcm9041126.

Dexter, F., Parra, M.C., Brown, J.R., Loftus, R.W., 2020. Perioperative COVID-19 Defense: An Evidence-Based Approach For Optimization of Infection Control and Operating Room Management. Anesth. Analg. in press https://doi.org/10.1213/ANE.00000000004829.

Eggers, M., Eickmann, M., Zorn, J., 2015. Rapid and effective virecidal activity of povi

Endevero Ab listed in Table 1, the \([\text{DiC}10]([\text{Cl})/\text{C12E8}/\beta-\text{CD}\) mixture (50 µM)

http://dx.doi.org/10.3762/bjoc.12.261.

Geller, C., Varbanov, M., Duval, R.E., 2012. Human coronaviruses: insights into en

https://doi.org/10.1213/ANE.00000000004829.

Garrido, P.F., Calvelo, M., Blanco-González, A., Veleuce, U., Suárez, F., Conde, D., Cabezon, A., Piñeiro, Á., Garcia-Fandino, R., 2020. The Lord of the NanoRings: cy

https://doi.org/10.1016/j.ejps.2010.06.017.

Kampf, G., Todt, D., Pfaender, S., Steinmann, E., 2020. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J. Hosp. Infect. 104, 246–251. https://doi.org/10.1016/j.jhin.2020.01.022.

Kärber, G., 1931. Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. Arch. Exp. Path. Pharmak 162, 480–487. https://doi.org/10.1007/BF01863914.

Leclercq, L., Tessier, J., Douyère, G., Nardello-Rataj, V., Schmitzer, A.R., 2020. Structure-activity relationships between cyclodextrins and didecyldimethylammonium chloride against enveloped viruses: Toward eco-biocidal formulations. Int. J. Pharm. 512, 273–281.

https://doi.org/10.1016/j.ijpharm.2016.08.057.

Leclercq, L., Leclercq, L., Nardello-Rataj, V., Aubry, J.-M., 2010. Structure-activity relationship of cyclodextrin/biocidal double-tailed ammonium surfactant host-guest complexes: towards a delivery molecular mechanism? Eur. J. Pharm. Sci. 41, 265–275. https://doi.org/10.1016/j.ejps.2010.06.017.

Leclercq, L., Leclercq, L., Aubry, J.-M., Nardello-Rataj, V., Schmitzer, A.R., 2020. Structure-activity relationships between cyclodextrins/biocidal double-tailed ammonium surfactant host-guest complexes: towards a delivery molecular mechanism? Eur. J. Pharm. Sci. 41, 265–275. https://doi.org/10.1016/j.ejps.2010.06.017.

Leclercq, L., Tessier, J., Douyère, G., Nardello-Rataj, V., Schmitzer, A.R., 2020. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J. Hosp. Infect. 104, 246–251. https://doi.org/10.1016/j.jhin.2020.01.022.

Kärber, G., 1931. Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. Arch. Exp. Path. Pharmak 162, 480–487. https://doi.org/10.1007/BF01863914.

Leclercq, L., 2016. Interactions between cyclodextrins and cellular components: Towards greener medical applications? Beilstein J. Org. Chem 12, 2644–2662. https://doi.org/10.3762/bjoc.12.261.

Leclercq, L., Leclercq, L., Nardello-Rataj, V., Aubry, J.-M., 2010. Structure-activity relationship of cyclodextrin/biocidal double-tailed ammonium surfactant host-guest complexes: towards a delivery molecular mechanism? Eur. J. Pharm. Sci. 41, 265–275. https://doi.org/10.1016/j.ejps.2010.06.017.

Leclercq, L., Nardello-Rataj, V., Rauwel, G., Aubry, J.-M., 2010. Structure-activity relationship of cyclodextrin/biocidal double-tailed ammonium surfactant host-guest complexes: towards a delivery molecular mechanism? Eur. J. Pharm. Sci. 41, 265–275. https://doi.org/10.1016/j.ejps.2010.06.017.

Leclercq, L., Leclercq, L., Aubry, J.-M., Nardello-Rataj, V., Schmitzer, A.R., 2020. Persistence of coronaviruses on inanimate surfaces and their inactivation with biocidal agents. J. Hosp. Infect. 104, 246–251. https://doi.org/10.1016/j.jhin.2020.01.022.

Kärber, G., 1931. Beitrag zur kollektiven behandlung pharmakologischer reihenversuche. Arch. Exp. Path. Pharmak 162, 480–487. https://doi.org/10.1007/BF01863914.
Lu, C.-W., Liu, X.-F., Jia, Z.-F., 2020. 2019-nCoV transmission through the ocular surface must not be ignored. Lancet Lond. Engl. 395, e39. https://doi.org/10.1016/S0140-6736(20)30313-5.

Malkin, A.J., McPherson, A., Geroush, P.D., 2003. Structure of intracellular mature vacinia virus visualized by in situ atomic force microscopy. J. Virol. 77, 6332-6340. https://doi.org/10.1128/jvi.77.11.6332-6340.2003.

Nardello-Rataj, V., Leclercq, L., 2016. Aqueous solutions of didecyldimethylammonium chloride and octethyleneglycolmonododecyl ether: Toward synergistic formulations against enveloped viruses. Int. J. Pharm. 511, 550-559. https://doi.org/10.1016/j.ijpharm.2016.07.045.

Ohtani, M., Irie, T., Uekama, K., Fukunaga, K., Pitha, J., 1989. Differential effects of α-, β- and γ-cyclodextrins on human erythrocytes. Eur. J. Biochem. 186, 17–22. https://doi.org/10.1111/j.1432-1033.1989.tb15171.x.

Peng, X., Xu, X., Li, Y., Cheng, L., Zhou, X., Ren, B., 2020. Transmission routes of 2019-nCoV and controls in dental practice. Int. J. Oral Sci. 12, 9 e4168-020-0075-9.

Perlman, S., Netland, J., 2009. Coronavirus post-SARS: update on replication and pathogenesis. Nat. Rev. Microbiol. 7, 439–450. https://doi.org/10.1038/nrmicro2147.

Pratelli, A., 2007. Action of disinfectants on canine coronavirus replication in vitro. Zoonoses Public Health 54, 383–386. https://doi.org/10.1111/j.1863-2378.2007.01079.x.

Pratelli, A., Colao, V., 2015. Role of the lipid rafts in the life cycle of canine coronavirus. J. Gen. Virol. 96, 331–337. https://doi.org/10.1099/vir.0.07870-0.

Prince, H.N., Prince, D.L., 2001. Principles of viral control and transmission. In: Block, S.S. (Ed.), Disinfection, Sterilization, and Preservation, 5th Ed. Lippincott Williams & Wilkins, Philadelphia, PA, U.S.A., pp. 548.

Quatiere, L.F., Pearson, G.R., 1979. Epstein-Barr virus-induced membrane antigens: immunochemical characterization of Triton X-100 solubilized viral membrane antigens from EBV-superinfected Raji cells. Int. J. Cancer. 23, 808-417. https://doi.org/10.1002/ijc.2910230612.

Rauwel, G., Leclercq, L., Cricquelet, J., Aubry, J.-M., Nardello-Rataj, V., 2012. Aqueous mixtures of di-n-decyl(dimethylammonium chloride/polyoxyethylene alkyl ether: dramatic influence of tail/tail and head/head interactions on co-micellization and biocidal activity. J. Colloid Interface Sci. 374, 176–186. https://doi.org/10.1016/j.jcis.2012.02.006.

Salmon, B., Cunningham, C., Davison, A.J., Harris, W.J., Baines, J.D., 1998. The herpes simplex virus type 1 UL17 gene encodes virion tegument proteins that are required for caggregation and packaging of viral DNA. J. Virol. 72, 3779–3788. https://doi.org/10.1128/JVI.72.5.3779-3788.1998.

Sattar, S.A., Springthorpe, V.S., Karim, Y., Loro, P., 1989. Chemical disinfection of non-porous inanimate surface experimentally contaminated with four human pathogenic viruses. Epidemiol. Infect. 102, 493-505. https://doi.org/10.1017/ S0950268800070211.

Schrann, C.L., Minbiolo, K.P.C., Wuest, W.M., 2020. Are Quaternary Ammonium Compounds, the Workhorse Disinfectants, Effective against Severe Acute Respiratory Syndrome-CoV-2? ACS Infect. Dis. 6, 1553–1557. https://doi.org/10.1021/acsinfecdis.0c00265.

Shi, B., Xia, Z., Xiao, S., Huang, C., Zhou, X., Xu, H., 2020. Severe Pneumonia due to SARS-CoV-2 and Respiratory Syncytial Virus Infection: A Case Report. clinical pediatrics https://doi.org/10.1077/009922820902016.

Sperman, C., 1998. The method of right and wrong cases (constant stimuli) without Gauss’s formulae. Br. J. Psychol. 2, 227–242. https://doi.org/10.1111/j.2044-8295.1908.tb00176.x.

Tang, Y.W., Crowe Jr, J.E., 2007. Respiratory syncytial virus and human metapneumovirus. In: Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L., Pfifer, M.A. (Ed.), Manual of clinical microbiology, 9th ed. ASM Press, Washington, USA, pp. 1361–1372.

To, K.K.-W., Tang, O.T.-Y., Chak-Yan Yip, C., Chan, K.-H., Wu, T.-C., Chan, J.M.C., Leung, W.-S., Chik, T.S.-H., Choi, C.Y.-C., Kandambly, D.H., Lung, D.C., Tam, A.R., Poon, R.W.-S., Fung, A.Y.-F., Hung, I.F.-N., Cheng, V.C-C., Chan, J.F.-W., Yuen, K.-Y., 2020. Consistent detection of 2019 novel coronavirus in saliva. Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. https://doi.org/10.1093/cid/ciaa1419. ciaa149.

Tobe, S., Majima, T., Tadenuma, H., Suekuni, T., Sakai, K., Sakai, H., Abe, M., 2015. Nonionic Surfactants Enhancing Bacterial Activity at Their Critical Micelle Concentrations. J. Oleo Sci. 64, 61–68. https://doi.org/10.5650/jos.xx14159.

Ulley, T.J., Ducharme, N.A., Varathakivi, V., Shepherd, B.E., Santangelo, P.J., Lindquist, M.E., Goldenring, J.R., Crowe Jr, J.E., 2008. Respiratory syncytial virus uses a Vps4-independent budding mechanism controlled by Rab11-FIP2. Proc. Natl. Acad. Sci. USA 105, 10209–10214. https://doi.org/10.1073/pnas.0712144105.

Wallace, K.B., Khan, M.A., Carlson, R.M., Rice, S., Froberg, M.K., 2003. Cyclodextrin compositions and methods of treating viral infections. U.S. Patent 0 20030612.

Whitworth, J., 2020. COVID-19: a fast evolving pandemic. Trans. R. Soc. Trop. Med. Hyg. 114, 241–248. https://doi.org/10.1093/trstmh/traa025.

World Health Organization (WHO), 1993. Disinfection and Sterilization. Laboratory Biosafety Manual, 2nd ed. World Health Organization (WHO), Geneva, pp. 60–70.

Zana, R., 1996. Critical micellization concentration of surfactants in aqueous solution and free energy of micellization. Langmuir 12, 1208–1211. https://doi.org/10.1021/ la950691q.

Zhang, L., Liu, Y., 2020. Potential interventions for novel coronavirus in China: A systematic review. J. Med. Virol. 92, 479–490. https://doi.org/10.1002/jmv.25707.

Zwart Voorspuij, A.J., Nass, C.A., 1957. Some aspects of the notions additivity, synergism and antagonism in the simultaneous activity of two antibacterial agents in vitro. Arch. Int. Pharmacody. Ther. 109, 211–228.