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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 ($[DiC_{10}][Cl]$), dodecyloctaglycol ($C_{12}E_8$), 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 ($[DiC_{10}][Cl]$), 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 ($C_{12}E_8$), making the detergent-disinfectant combination ideal for general sanitation purposes (Rauwel et al., 2012). As $C_{12}E_8$ is able to solubilize the viral envelope via micellization, the $C_{12}E_8$ potentiates the virucidal activity of $[DiC_{10}][Cl]$ 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 $[DiC_{10}][Cl]$ against enveloped viruses since both of them act on the viral-lipids (Leclercq et al., 2016).

It may be hypothesized that $[DiC_{10}][Cl]/C_{12}E_8$/CD systems would demonstrate synergistic effects against enveloped viruses as these

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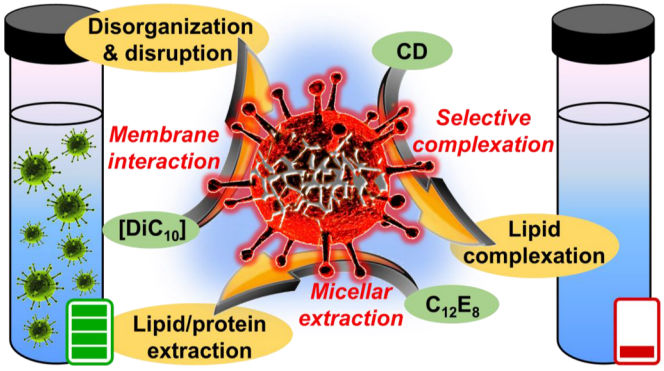

Fig. 1. Effects of didecyldimethylammonium cation, [DiC_{10}], dodecyloctaglycol, $C_{12}E_8$, and native cyclodextrins, CDs, on the lipid membrane of enveloped viruses.

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 ([DiC_{10}][Cl]) was as synthesized according to the procedure described in our previous work (Leclercq et al., 2010). Dodecyloctaglycol ($C_{12}E_8$) was purchased from TCI (purity higher than 99.9%). The other chemical compounds were purchased from Sigma-Aldrich Chemical at the highest purity available. The compounds used in the biological assays, strains and cells 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, 30 mL; sodium thiosulfate (5 H_2O), 5 g; histidine chlorhydrate, 1 g; saponin, 30 g; tryptone salt, 9.5 g; water qs. 1 L. Experiments were assayed in triplicate.

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 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 Doses, $TCID_{50}$, Spearman, 1908; Kärber, 1931). The detection limit was 5.62 $TCID_{50}$/mL. Virus stocks were 1.3 × 10^7 $TCID_{50}$/mL for RSV, 1.5 × 10^7 $TCID_{50}$/mL for HSV-1, 1.1 × 10^7 $TCID_{50}$/mL for VACV, and 6 × 10^6 $TCID_{50}$/mL for CVB4.

2.3. Virucidal assay

200 µL of the viral stock solution was added to 200 µL of surfactant ($C_{12}E_8$ and/or [DiC_{10}][Cl]) or 200 µL of the aqueous ternary mixtures of CD, $C_{12}E_8$ and [DiC_{10}][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-log_{10} was regarded as evidence of sufficient virucidal activity according to EN 14476. The lowest concentration giving a reduction in virus titer of ≥4-log_{10} was the minimum virucidal concentration (MVC).

2.4. Critical micelle concentration

Various solutions of [DiC_{10}][Cl], $C_{12}E_8$ 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 (D_h) 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 D_h ± 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 (α ± 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 par 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 par 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 than 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, [DiC_{10}][Cl] was able to reduce the viral loading of canine CoV by > 4.0 $\log_{10}$ but benzalkonium 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; Asculai 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 [DiC_{10}][Cl]/$C_{12}E_8$/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 [DiC_{10}][Cl] (*Q*), $C_{12}E_8$ (*E*), and CD (*α, 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 [DiC_{10}][Cl] and $C_{12}E_8$ after 15 min of contact time. For [DiC_{10}][Cl], MVC is lower than the CMC (1,200 μM; see Table 2). As previously reported this observation supports the insertion of free [DiC_{10}] cation within the viral envelope leading to membrane disorganization until destruction and virus inactivation (Leclercq et al., 2016). The opposite holds for $C_{12}E_8$: the MVC value is higher than the CMC of the surfactant (125 *vs.* 100 μM; see Table 2) which suggests that the $C_{12}E_8$ micelles are the active species (Nardello-Rataj and Leclercq, 2016). It is noteworthy that the hydrodynamic diameter (D_h) 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 $C_{12}E_8$ insertion in the outer membrane associated with a rapid flip-flop across the lipid membrane leading to the

Table 1
Dose-dependent virucidal activity and corresponding synergy index (*SI*) against RSV (enveloped RNA virus).[a]

	Concentration of each component (μM)								*SI*[b]
	20	35	50	75	125	250	500	1000	
(-), α, β or γ	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-
Q	≤1.0	≤1.0	2.0	3.2	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	-
Q/α	≤1.0	≤1.0	≤1.0	1.7	3.3	**≥6.5***	**≥6.5**	**≥6.5**	2.0
Q/β	≤1.0	≤1.0	≤1.0	1.2	2.1	**≥6.5***	**≥6.5**	**≥6.5**	2.0
Q/γ	≤1.0	1.2	3.5	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.6
E	≤1.0	1.1	2.0	3.6	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	-
E/α	≤1.0	≤1.0	≤1.0	1.2	2.9	**≥6.5***	**≥6.5**	**≥6.5**	2.0
E/β	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	3.1	**≥6.5***	**≥6.5**	4.0
E/γ	≤1.0	1.2	2.1	3.8	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	1.0
Q/E	1.2	2.7	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.8
Q/E/α	1.6	3.2	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.8
Q/E/β	2.0	**4.2***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.6
Q/E/γ	1.9	**4.0***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.6

[a] Virucidal activity in $\log_{10}$ titer reduction factor recorded after 15 min of contact time at room temperature with an initial RSV of 1.3×10^7 $TCID_{50}$/mL (with *α* = α-CD, *β* = β-CD, γ = γ-CD, *Q* = $[DiC_{10}][Cl]$ and *E* = $C_{12}E_8$).
[b] Calculated according equation 1.
* Minimum virucidal concentration (MVC) = the lowest concentration able to inactivate at least 99.99% of viruses.

extraction of lipids above the CMC. In contrast, native CDs are not able to induce a virucidal activity against RSV at low concentrations (<1,000 μM) after 15 min or 2 h of contact time. However, it is noteworthy that a reduction in virus titer of ≥4-$\log_{10}$ after 24h was obtained for β-CD at 6,000 μM. This result is close to the value observed against HSV-1 (Wallace et al., 2003).

A second series of control experiments was carried out in the presence of native CDs with $[DiC_{10}][Cl]$ or $C_{12}E_8$. It is noteworthy that α-, β- and γ-CD increase the CMC of $[DiC_{10}][Cl]$ and $C_{12}E_8$ due to the competitive inclusion of the surfactants into the CD cavity (Table 2). However, even if CDs are known to bind surfactant molecules below the CMC, no interactions of CDs with the surfactant micelles (above the CMC) were observed in this work (Nardello-Rataj and Leclercq, 2016). Indeed, the hydrodynamic diameter (see D_h in Table 2) of the micelles remains unchanged with or without CDs. In addition, for $[DiC_{10}][Cl]$, the ζ-potentials as well as the degrees of ionization of micelle (*α*) were not influenced by the presence of CDs (Table 2). These findings confirm that both inclusion complexes and CDs remain in solution and are not in the micelle shell. Since the virucidal activity of $[DiC_{10}][Cl]$ or $C_{12}E_8$ can be potentiated, the synergy index (*SI*) was estimated using the following equation:

$$SI = \sum \frac{[X]}{MVC_X} \quad (1)$$

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* or *CD*). It is noteworthy that for CD, the $[CD]/MVC_{CD}$ is negligible with respect to the others as MVC_{CD} 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 $[DiC_{10}][Cl]$/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 $[DiC_{10}][Cl]$/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 $[DiC_{10}][Cl]$/γ-CD mixture is synergetic (*SI* = 0.6, Table 1). The two other $[DiC_{10}][Cl]$/CD combinations result in antagonistic responses. Therefore, the virucidal efficiency is also linked to the $[DiC_{10}][Cl]$/CD binding constants that increases in the order γ-CD < β-CD < α-CD (Table 2, Funasaki et al., 2000). As a given free $[DiC_{10}]$ 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 $[DiC_{10}]$ 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 $[DiC_{10}][Cl]$/CD mixtures is more linked to the equilibrium constants, rather than the affinity of CDs for lipids. For $C_{12}E_8$/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 $C_{12}E_8$/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 $[DiC_{10}][Cl]$ and $C_{12}E_8$. The CMC of mixed surfactant (60 μM) was sharply lower than that of sole surfactants (1,200 and 100 μM for $[DiC_{10}][Cl]$ and $C_{12}E_8$, see Table 2) due to favorable interactions between the components in the

Table 2
Physicochemical parameters of $[DiC_{10}][Cl]/C_{12}E_8$ (*Q/E*) mixed systems with or without native cyclodextrins (*α, β* and γ) in aqueous solution at 25°C compared to $[DiC_{10}][Cl]$ and $C_{12}E_8$ alone.[a]

	Micelles properties[b]				Binding constant (M^{-1})[c]			
	CMC (μM)	D_h (nm)	ζ (mV)	α (%)	K^Q_1	K^Q_2	K^E_1	K^E_2
Q	1,200[d]	4.0	57.9	61	-	-	-	-
Q/α	5,500[d]	3.9	60.4	60	26,000[d]	7,500[d]	-	-
Q/β	5,100[d]	4.1	54.9	63	9,700[d]	2,900[d]	-	-
Q/γ	10,200[d]	4.0	61.7	58	6,860[d]	-	-	-
E	100[d]	7.9	0	-	-	-	-	-
E/α	160	8.1	0	-	-	-	7,300[d]	2,400[d]
E/β	300	7.7	0	-	-	-	17,000[d]	-
E/γ	105	8.0	0	-	-	-	70[d]	-
Q/E	60[d]	8.5	35.4	73	-	-	-	-
Q/E/α	90	8.7	38.7	75	26,000[d]	7,500[d]	7,300[d]	2,400[d]
Q/E/β	80	8.6	40.1	76	9,700[d]	2,900[d]	17,000[d]	-
Q/E/γ	70	8.4	34.2	72	6,860[d]	-	70[d]	-

[a] All binary or ternary systems are equimolar.
[b] CMC = critical micelle concentration, D_h = hydrodynamic diameter, ζ-potential recorded at 10 × CMC, *α* = the degree of ionization of the micelle.
[c] Determined from surface tension modelling using single surfactant/cyclodextrin systems in aqueous solution with dilution experiments.
[d] Taken in Leclercq et al., 2013.

co-micelles. On the other hand, according to the data reported in Table 1, the $[DiC_{10}][Cl]$ and $C_{12}E_8$ mixture is synergetic (SI = 0.6). As the MVC value (100 μM, Table 1) is clearly much higher than the CMC of $[DiC_{10}][Cl]/C_{12}E_8$ 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-$\log_{10}$ reduction at 50 μM of $[DiC_{10}][Cl]$ and 50 μM of $C_{12}E_8$, only of 1-$\log_{10}$ reduction was observed for CVB4 (non-enveloped virus) with the $[DiC_{10}][Cl]/C_{12}E_8$ 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 $C_{12}E_8$ micelles but higher than those of the pure $[DiC_{10}][Cl]$ micelles (compare the hydrodynamic diameters, D_h, in Table 2). This observation supports that the co-micellization is governed by the $C_{12}E_8$ molecules. Secondly, the ζ-potential of co-micelles was weaker than that of the $[DiC_{10}][Cl]$ micelles whereas the degree of co-micelle ionization (α) was higher. These findings are due to the "podand" effect of the polyoxyethylene chains which can be seen as an open-chain equivalent to a crown ether able to complex $[DiC_{10}]$ cations (Rauwel et al., 2012). This cation binding podand decreases the electrostatic repulsion between the $[DiC_{10}]$ 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 $C_{12}E_8$ micelles improves the probability of the attachment of $[DiC_{10}]$ 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 $[DiC_{10}]$ cations in mixed micelles will increase the adsorption of $[DiC_{10}]$ 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 $[DiC_{10}][Cl]/C_{12}E_8$/CD ternary mixtures were clearly lower than that of sole surfactants (1,200 and 100 μM for $[DiC_{10}][Cl]$ and $C_{12}E_8$, 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 $[DiC_{10}][Cl]/C_{12}E_8$/CD ternary mixtures 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:

$$\Delta G^{\circ}_{mic} = RT \ln(CMC) \quad (2)$$

In all cases, the observed CMCs of $[DiC_{10}][Cl]/C_{12}E_8$/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 $[DiC_{10}][Cl]$ surfactants. Therefore, the stability of the $[DiC_{10}][Cl]/C_{12}E_8$/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 $[DiC_{10}][Cl]/C_{12}E_8$/CD ternary system and the resulting formulations exhibit a very long-term thermodynamic stability. In term of virucidal activity against RSV, the $[DiC_{10}][Cl]/C_{12}E_8$/CD mixture ternary mixtures were synergetic. However, the mixtures that use β- and γ-CD (SI = 0.6) were more synergistic 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 $[DiC_{10}][Cl]/$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 $[DiC_{10}][Cl]/$γ-CD binary mixture is only disinfectant where the $[DiC_{10}][Cl]/C_{12}E_8/$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 $[DiC_{10}]/C_{12}E_8$ (envelope disorganization and lipids extraction) and CDs (lipids complexation) 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 $[DiC_{10}]/C_{12}E_8$ co-micelles, (*ii*) the adsorption at the membrane lipids interface, (*iii*) the fast insertion/removal between the $[DiC_{10}]$ 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 $[DiC_{10}]$ cations interact, penetrate and weaken the viral envelope due to the multiple equilibria in $[DiC_{10}][Cl]/C_{12}E_8$/CD ternary systems also take place (see Fig. 2). Indeed, next to the $[DiC_{10}]/C_{12}E_8$ co-micellization equilibrium, the introduction of $C_{12}E_8$ in $[DiC_{10}][Cl]$/CD system results in the reduction of the CD's affinity for $[DiC_{10}]$ due to the $C_{12}E_8$ 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 $[DiC_{10}]$ cations are then obtained. In the presence of viruses, the free $[DiC_{10}]$ cation can be interacted with the viral envelope. Due to the insertion in the envelope, the free $[DiC_{10}]$ concentration decreases, altering the equilibria, so more $[DiC_{10}]$ cation and CD are released. At this stage, CDs are able to extract lipid from the viral envelope by complexation thus creating "holes". Consequently, in this mechanism

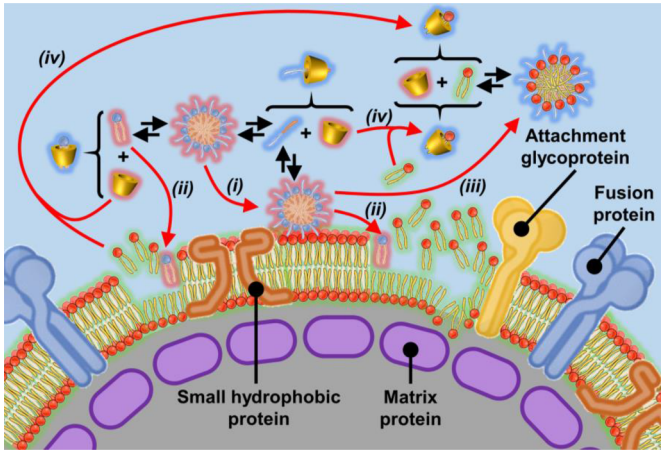

Fig. 2. Molecular virucidal mechanism of $[DiC_{10}][Cl]/C_{12}E_8$/CD mixtures against RSV: (*i*) adsorption of $[DiC_{10}]/C_{12}E_8$ co-micelles through electrostatic interaction with the viral envelope, (*ii*) insertion of $[DiC_{10}]$ in the envelope, (*iii*) micellar extraction of lipids, and (*iv*) lipids complexation by CDs.

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*).[a]

		Concentration of each component (μM)								*SI*[b]
		20	35	50	75	125	250	500	1000	
HSV-1	*(-), α, β* or γ	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-
	Q	≤1.0	1.1	1.5	3.7	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	-
	E	≤1.0	1.2	2.6	3.5	**≥6.5***	**≥6.5**	**≥6.5**	**≥6.5**	-
	Q/E	1.1	3.7	**5.7***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.8
	Q/E/α	≤1.0	2.2	**6.0***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.8
	Q/E/β	1.3	**4.0***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.6
	Q/E/γ	**4.1***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.3
VACV	*(-), α, β* or γ	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-
	Q	≤1.0	≤1.0	≤1.0	≤1.0	1.3	1.9	**≥6.5***	**≥6.5**	-
	E	≤1.0	≤1.0	≤1.0	≤1.0	1.2	1.7	**≥6.5***	**≥6.5**	-
	Q/E	≤1.0	1.2	2.8	**4.6***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.3
	Q/E/α	≤1.0	1.4	3.7	**4.9***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.3
	Q/E/β	≤1.0	1.3	**4.0***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.2
	Q/E/γ	≤1.0	1.2	**5.2***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	0.2
P. aeruginosa	*(-), α, β* or γ	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-
	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**	1.0
	Q/E/α	1.4	2.7	**4.5***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	1.0
	Q/E/β	≤1.0	2.1	**4.6***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	1.0
	Q/E/γ	1.2	2.1	**4.1***	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	**≥6.5**	1.0
C. albicans	*(-), α, β* or γ	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-
	Q	≤1.0	≤1.0	1.3	**4.3***	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	-
	E	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	≤1.0	-
	Q/E	≤1.0	**≥6.0***	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	0.5
	Q/E/α	≤1.0	**≥6.0***	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	0.5
	Q/E/β	≤1.0	**≥6.0***	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	0.5
	Q/E/γ	≤1.0	≤1.0	**4.6***	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	**≥6.0**	0.7

[a] Biocidal or virucidal activity in $\log_{10}$ titer reduction factor recorded at room temperature after 15 min of contact time for HSV-1 (1.5×10^7 $TCID_{50}$/mL), VACV (1.1×10^7 $TCID_{50}$/mL), *C. albicans* (1.6×10^6 CFU/mL) and 5 min for *P. aeruginosa* (1.0×10^8 CFU/mL) with α = α-CD, β = β-CD, γ = γ-CD, Q = $[DiC_{10}][Cl]$ and E = $C_{12}E_8$.

[b] 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.

the $[DiC_{10}]/C_{12}E_8$ co-micelles as well as the various $[DiC_{10}]$/CD inclusion complexes can be seen as reservoirs of $[DiC_{10}]$ and CDs which are readily available for interaction with the viral envelope (*i.e.* complexation and insertion, for CDs and $[DiC_{10}]$, 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 encephalitis whereas the VACV is used as model infections for human smallpox caused by the variola virus (VARV). As most of bacteria are Gram-negative, we worked with *Pseudomonas aeruginosa* an archetypal encapsulated rod-shaped pathogen that can cause disease in plants and animals, including humans. On the other hand, a commensal fungus which lives in the human mouth and gastrointestinal tract such as *Candida albicans* has been investigated because their overgrowth results in candidiasis that results in septicemia (Leclercq et al., 2020). It is noteworthy that according to the well-known disinfection scale, the pathogen susceptibility to chemical biocides is in the following order: enveloped viruses > bacteria > fungus > non-enveloped viruses (Prince and Prince, 2001).

For each pathogen, the biocidal (or virucidal) activity of $[DiC_{10}][Cl]$, $C_{12}E_8$ and CDs alone or in equimolar mixture has been determined (Table 3). $[DiC_{10}][Cl]$ or $C_{12}E_8$ alone were active against RSV and VACV, but CDs are inactive against both viruses. As with RNA viruses, synergistic effects were clearly observed for DNA viruses (HSV-1 and VACV): the *SI* values were comprised between 0.8 and 0.2. However, the extend of synergistic effect for $[DiC_{10}][Cl]/C_{12}E_8$/γ-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 $[DiC_{10}][Cl]/C_{12}E_8$/γ-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), $[DiC_{10}]/C_{12}E_8$ co-micelles and $[DiC_{10}]$ alone can interact and alter the viral envelope, after the insertion of $[DiC_{10}]$ cations, facilitating the lipid extraction by the CDs and leads to virus inactivation. We can therefore suppose that the required amount of inserted $[DiC_{10}]$ 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 $C_{12}E_8$ or CDs alone were inactive against *P. aeruginosa* and *C. albicans*, but $[DiC_{10}][Cl]$ was active against both pathogens. The $[DiC_{10}][Cl]/C_{12}E_8$ and $[DiC_{10}][Cl]/C_{12}E_8$/CD mixtures were also active against *P. aeruginosa* that $[DiC_{10}][Cl]$ alone. Therefore, only additive responses were obtained for binary and ternary mixtures (*SI* = 1, Table 3). In contrast, all the $[DiC_{10}][Cl]/C_{12}E_8$ and $[DiC_{10}][Cl]/C_{12}E_8$/CD combinations were synergetic against *C. albicans*. However, the extend of synergistic effect for $[DiC_{10}][Cl]/C_{12}E_8$/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 $[DiC_{10}][Cl]/C_{12}E_8$ 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 $[DiC_{10}][Cl]/C_{12}E_8$/β-CD mixture (50 μM of each) is able to fight RSV, HSV-1, VACV, *P. aeruginosa* and *C. albicans* whereas the equimolar $[DiC_{10}][Cl]/C_{12}E_8$ mixture (50 μM of each) is inactive on VACV and $[DiC_{10}][Cl]$ alone (50 μM) is only active against *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 $[DiC_{10}][Cl]$ alone and 2 products are up to 6 active ingredients (Health Canada, 2020a). $[DiC_{10}][Cl]$ is usually in mixture with other biocidal compounds: quaternary ammonium ($[DiC_8][Cl]$, $[C_8C_{10}][Cl]$, 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 $[DiC_{10}][Cl]$ alone ranges from 0.052 wt.% for ready-to-use (KLERICIDE CLEANROOM QUAT 450 by Ecolab) to 80% (ACTICIDE DDQ 80 by Thor Specialties Inc.; Health Canada, 2020a). For the mixtures, the lowest $[DiC_{10}][Cl]$ concentration is found to be 0.0083 wt.% supplemented with 0.0184, 0.0055 and 0.0138 wt.% of benzalkonium chloride, $[DiC_8][Cl]$and $[C_8C_{10}][Cl]$, respectively (BARDAC 205M-RTU by Lonza LLC; Health Canada, 2020a). All these products kill bacteria (*e.g. P. aeruginosa, S. aureus* and *E. coli*), viruses (*e.g.* HSV-1, HSV-2, Influenza A2 and VACV), and fungi (*e.g. C. albicans* and *T. mentagrophytes*). As depicted in Tables 1 and 3, the $[DiC_{10}][Cl]/C_{12}E_8$/β-CD ternary mixture is active against viruses (RSV, HSV-1 and VACV), bacterium (*P. aeruginosa*) and fungus (*C. albicans*) with only 0.0018, 0.0027 and 0.0057 wt.% of $[DiC_{10}][Cl]$, $C_{12}E_8$ and β-CD, respectively. Therefore, the composition of this new formulation is clearly reduced compared to the commercial ones. Indeed, a noticeable reduction of the active virucide ($[DiC_{10}][Cl]$) (by a factor of 4.6) as well as of the total active ingredients (by a factor of 4.5) is observed compared to BARDAC 205M-RTU used in home, hospital, institutions and industries. In addition, a partial replacement of a significant amount of the biocide by the eco- and bio-compatible cheapest β-CD whilst maintaining excellent activity is of great interest in the context of new efficient and eco-friendly biocidal mixtures. However, before use as disinfectant and cleaning product for use in public, institutional and household spaces, some steps remain essential such as the determination of the virucidal and biocidal activities in the presence of serum contamination. Moreover, the efficiency against SARS-CoV-2 requires probably higher concentration in active ingredients. Indeed, in the Bardac 205M family only the products containing at least 1.35, 3.0, 0.90 and 2.25 wt.% of $[DiC_{10}][Cl]$, benzalkonium chloride, $[DiC_8][Cl]$and $[C_8C_{10}][Cl]$, respectively (Bardac 205M-7.5 by Lonza LLC), are active against SARS-CoV-2 as indicated in the list of hard-surface disinfectants effective against this human CoV provided by Health Canada (2020b). However, if we succeed in the shift to concrete applications, our work could have a global impact in the prevention against known and new emerging viruses.

4. Conclusion

We have demonstrated that $[DiC_{10}]$ cations associated with $C_{12}E_8$ and native CDs show synergistic action against enveloped viruses (RSV, HSV-1 and VACV) and fungi (*C. albicans*), with additive responses against bacteria (*P. aeruginosa*). Indeed, these mixtures act as much more efficient virucide and biocide than each compound alone due to: (*i*) the formation of $[DiC_{10}][Cl]/C_{12}E_8$ co-micelles, (*ii*) the modification of membrane lipids interface, (*iii*) the fast insertion/removal between the $[DiC_{10}]$ cations and the lipids, and (*iv*) the alteration of membrane properties facilitating the lipid extraction by the CDs. Modification of lipid interactions allows the virus inactivation or cellular death by the exposure of the genome. As this synergistic effect, based on complex interactions and multiple equilibria, provides broad-spectrum eradication (enveloped viruses, bacteria and fungi), it is unlikely to lead to the development of pathogens resistance against these three components mixtures. These highly relevant results, in the current pandemic situation, support that this synergistic effect provides elements valuable for prevention of respiratory illnesses due to enveloped viruses, including SARS-CoV-2, and other hazardous pathogens in households and community settings. Work is under way to extend the concept of these systems to other disinfectants.

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