Transparent Polymeric Formulations Effective against SARS-CoV-2 Infection

Valentina Gentili, Daniele Pazzi, Sabrina Rizzo, Giovanna Schiuma, Edoardo Marchini, Stefania Papadia, Andrea Sartorel, Dario Di Luca, Francesca Caccuri, Carlo Alberto Bignozzi,* and Roberta Rizzo*

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ABSTRACT: The main route of the transmission of the SARS-CoV-2 virus is through airborne small aerosol particles containing viable virus as well as through droplets transmitted between people within close proximity. Transmission via contaminated surfaces has also been recognized as an important route for the spread of SARS-CoV-2 coronavirus. Among a variety of antimicrobial agents currently in use, polymers represent a class of biocides that have become increasingly important as an alternative to existing biocidal approaches. Two transparent polymeric compounds, containing silver and benzalkonium ions electrostatically bound to a polystyrene sulfonate backbone, were synthesized, through simple procedures, and evaluated for their antimicrobial properties against Gram-positive and Gram-negative bacteria and of the fungal species Candida albicans (ISO EN 1276) and for their antiviral activity toward 229E and SARS-CoV-2 coronaviruses (ISO UNI EN 14476:2019). The results showed that the two tested formulations are able to inhibit the growth of (1.5−5.5) \times 10^{11} CFU of Gram-positive bacteria, Gram-negative bacteria, and of the fungal species Candida albicans. Both compounds were able to control the 229E and SARS-CoV-2 infection of a target cell in a time contact of 5 min, with a virucidal effect from 24 to 72 h postinfection, according to the European Medicines Agency (EMA) guidelines, where a product is considered virucidal upon achieving a reduction of 4 logarithms. This study observed a decrease of more than 5 logarithms, which implies that these formulations are likely ideal candidates for the realization of transparent surface coatings that are capable of maintaining remarkable antibacterial activity and SARS-CoV-2 antiviral properties over time.

KEYWORDS: benzalkonium, silver, SARS-CoV-2, antiviral, antibacterial

1. INTRODUCTION

The global COVID-19 pandemic highlighted the need for innovative methods and technologies to mitigate the spread of viruses. The main route of the transmission of the SARS-CoV-2 virus occurs through small, airborne aerosol particles containing viable virus as well as through larger droplets that are transmitted between people within close proximity. Transmission via contaminated surfaces has also been recognized as an important route for the spread of SARS-CoV-2 coronavirus, and several approaches to produce antimicrobial surfaces have been recently explored.1,2

Notable results have been obtained with nanostructured surfaces,3,4 surfaces coated with copper oxide,5,6 and surface coated with cuprous oxide particles bound with polyurethane.8

Among a variety of available antimicrobial agents, polymers represent a class of biocides that have become increasingly important as an alternative to existing biocides. Conventional disinfectants are based on low-molecular-weight liquids or gases, so their use and duration are restricted by their volatility. Antimicrobial polymers can be tethered to surfaces enabling the killing of microbes without releasing biocides. Many of the previously reported antimicrobial polymers have focused on antibacterial capabilities; however, there has been much less focus on antiviral properties. Antimicrobial polymers have been known since 1965, when Cornell and Donaruma described polymers and copolymers that kill bacteria.9 Since then, a number of reports appeared in the literature describing bactericidal coatings incorporating bound10 or unbound biocidal agents, such as metal-oxide particles,10 chlorine dioxide,11 iodine compounds,12,13 silver nanoparticles,14 benzophenone,15 or quaternary ammonium (QA) compounds16,17 mixed with a polymeric binder. Among these coatings, QA compounds are probably the most widely used antibacterial agents for medical and public health applications because they have been shown to

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be effective against both Gram-negative and Gram-positive bacteria.20,21

According to the literature,19 there are three general types of antimicrobial polymer: (1) polymeric biocides, where the repeating unit is a bioicide; (2) biocidal polymers, where the active unit is randomly embedded within a macromolecule, and (3) biocide-releasing polymers where the polymeric backbone acts as a carrier support for the biocides that can be transferred to the cell. In addition to these classic polymers, photoactive polymers and oligomers have recently demonstrated highly effective light-induced inactivation of SARS-CoV-2, which may cause a 5-log reduction in pfu/mL within 10 min of irradiation.20

Our work has focused on simple polymeric compounds where an active principle can be electrostatically bound to the backbone of a water-soluble anionic polymer, resulting in the formation of water-insoluble polymeric materials, which can be then solubilized in alcoholic solvents. In this first study, we have considered silver-containing species and benzalkonium ions electrosodically bound to polystyrene sulfonate. The alcoholic solutions of these polymeric materials are ideal for realizing antimicrobial coatings on everyday items handled by people such as doorknobs, keyboards, handles, as well as on any type of filtration system where the polymeric backbone adheres to the solid substrate because of significant van der Waals interactions. The resulting films are transparent and are expected to exhibit a long-lasting antimicrobial effect.

The observation that the coated materials are highly effective against Gram-positive and Gram-negative bacteria and Candida albicans prompted us to investigate the antiviral activity of the solid polymeric film because viruses can also survive for a period of hours to several days once established on inanimate surfaces. We report here strong evidence of the activity of polymeric films against 229E and SARS-CoV-2 coronaviruses.

2. MATERIALS AND METHODS

2.1. Materials.
All chemical products were purchased from Sigma-Aldrich. Elemental analysis C, H, N was performed with a LECO CHN analyzer. Silver was determined through plasma atomic absorption spectrometry with a Perkin Elmer Optima 3100 XL. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker Vertex 70 FT-IR in diffuse reflectance mode dispersing the chemical powder in KBr (potassium bromide). Dimensional analysis and zeta potential were obtained with a Z-sizer Malvern instrument.

2.2. Preparation.
The preparation of the silver complex, complex salts, and micellar derivatives was first described in the patent application PCT/EP2020/060686.

\[ \text{Na}_3\text{Ag(HPA)}_2 \cdot 2\text{H_2O} \]

Na\text{[Ag(MPA)]_2} (MPA = 2-Mercapto-4-methyl-5-thiazolacetic). To a 1.135 g amount of the protonated MPA (2-mercapto-4-methyl-5-thiazolacetic acid, Aldrich), 12.36 g of 1 M NaOH solution in water was added. Distilled water (186.5 g) was then added, and the solution was kept stirring for 5 min. An aqueous solution (100 g) containing 0.51 g of \text{AgNO}_3 was finally added, and the yellow solution was kept under stirring for an additional 30 min. The Na\text{[Ag(MPA)]_2} complex salt was precipitated by the addition of an excess of acetone, filtered, and air-dried; \text{Ag+} % = 0.108%. Elemental analysis: calculated for \text{Na}_3\text{AgCl}_2\text{N}_3\text{S}_4\text{O}_8\text{H}_{10}\text{O}: C 26.14; N 5.08; H 1.83; Ag 19.57. Found: C 25.87; N 5.0; H 1.8; Ag 19.46.

[Ag(MPA)]_2 \cdot \text{H_2O} (Bz = Benzalkonium) Micellar Derivatives. Benzalkonium (Bz) CAS. n. 63,449–41-2 is a mixture of alkylbenzylidinemethyammonium chlorides, in which the alkyl group has various even-numbered alkyl chains from 8 to 18. The average molecular weight of the chloride salt is 370 g. Uncharged [Ag(MPA)]_2 (Bz) salt can be easily precipitated by the addition of a slight excess of benzalkonium chloride to an aqueous solution of the silver complex Na\text{[Ag(MPA)]_2}.

The addition of an excess of benzalkonium chloride results, instead, in the dissolution of [Ag(MPA)]_2 (Bz) because of the formation of positively charged micellar derivatives with a size distribution in the range 10–500 nm (see the next section).

Polymeric salts PSS-Bz-Ag and PSS-Bz. Polymeric salts of the positively charged micellar derivatives of [Ag(MPA)]_2 (Bz) or Bz ions were obtained in distilled water by the addition of sodium polystyrene sulfonate (PSS). The precipitated neutral polymers containing PSS, Ag(MPA)_2, and Bz moieties (PSS-Bz-Ag) or PSS and Bz ions (PSS-Bz) were separated from the aqueous solutions by filtration, washed with water, and dried at 50 °C. The polymeric solid was then dissolved in isopropanol or ethanol to obtain a solution of PSS-Bz-Ag containing Bz 1.25%, PSS 0.62%, and Ag 0.0025% and a solution of PSS-Bz containing Bz 1.25% and PSS 0.62%.

2.3. Bacterial Strains. The antimicrobial activity of the liquid products PSS-Bz-Ag (composition: Bz 1.25%, Ag+ 0.0025%, PSS 0.62%, ethanol) and PSS-Bz (composition: Bz 1.25%, PSS 0.62%, ethanol) was tested against the following strains of microorganisms (purchased from Diagnostic International Distribution S.p.A.): Pseudomonas aeruginosa ATCC 15442, Staphylococcus aureus ATCC 6538, Escherichia coli ATCC 10536, Enterococcus hirae ATCC 10541, Candida albicans ATCC 10231, in accordance with ISO EN 1276. Microbial suspensions were prepared according to EN1276 from lyophilized pellets resuspended in the LB (Luria Broth) medium. The concentration was determined by spectrophotometry, horizontal method, and biochemical test. Microbial suspensions of the different strains were prepared with concentrations expressed as colony-forming units (CFU) comprised between 1.5 × 10^8 and 5.5 × 10^9 for each species. Samples (100 μL) of the two products were deposited in the center of Petri dishes and dried for 2 h in an oven at 37 °C. The microbial pool (100 μL) was then added and left in contact with PSS-Bz-Ag or PSS-Bz for 5 min. TSA (tryptone soya agar) was added after cooling at 40 °C and incubated at 37 °C for 24 h. Microbial titration was determined using the horizontal method. The experiments were performed in triplicate.

2.4. Cells and Viruses.
MRC5 human fibroblast cells (ATCC CCL171) and African green monkey epithelial kidney cells, VeroE6 (ATCC CRL1586), were grown in monolayers in Eagle’s minimal essential medium (MEM) with nonessential amino acids (Lonza Biosciences; Italy) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco-Life Technologies; Italy), penicillin (100 units/mL; Sigma-Aldrich), streptomycin (100 μg/mL; Sigma-Aldrich; Italy), and 1-glutamine (2 mM; Sigma-Aldrich; Italy).

Human coronavirus 229E (ATCC VR-740) and the clinical isolate of SARS-CoV-2, kindly provided by Prof. A. Caruso and Prof. F. Caccuri, University of Brescia, Italy, were propagated and titrated by qPCR assay on MRC5 or VeroE6 cells, respectively.24 All procedures were carried out in a biosafety level-3 (BSL-3) laboratory.

2.5. Cytotoxicity Control.
Cytotoxicity control was performed on MRC5 and VeroE6 cells. The culture medium was mixed with PSS-Bz-Ag, PSS-Bz, or PBS (phosphate-buffered saline, 1X control solution) (Gibco, USA). The resulting solutions were added to cell cultures and incubated for 5, 10, 15 min, 24, 48, and 72 h. Cell viability was examined by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) colorimetric assay (Roche Diagnostics Corporation, Indianapolis, IN) and trypan blue dye (Sigma-Aldrich; Italy) exclusion. 2% DMSO (dimethyl sulfoxide; Sigma-Aldrich; USA) was used as the positive control of cell cytotoxicity. The experiments were performed in triplicate.

2.6. Antiviral Assays.
The antiviral effect on the 229E and SARS-CoV-2 infection was evaluated by quantitative assays (plaque assay), in accordance with ISO UNI EN 14476:2019. The 229E and human coronavirus SARS-CoV-2 suspensions were prepared by infecting the monolayers of MRC5 and VeroE6 cells, respectively. The infections were performed at 1.0 multiplicity of infections (MOIs).20

On the day of testing, sterile Petri dishes (35 mm diameter) were prepared, as previously reported (Section 2.3). The viral suspension was added to the PSS-Bz-Ag, PSS-Bz, or PBS (control)-treated Petri dishes and incubated for 5 min at room temperature. The viral suspension was then harvested and incubated with susceptible cells for 1 h at 37 °C to let the viral particle infect the cells. The virus inoculum

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was then removed, the cells were washed, and 1 mL of EMEM 2% FCS was added. The ability of the viral particles to infect susceptible cells was assessed on cell culture supernatants 24, 48, and 72 h postinfection (p.i.). Viral titration was performed by plaque assay and quantitative reverse transcription (qRT)-PCR.

2.6.1. Plaque Assay. Cell culture supernatants were harvested and placed in an ice bath immediately. The samples were inoculated in cell cultures immediately, within 10 s. Five days after infection, cells were methanol-fixed, and plaques were stained with crystal violet (0.1%) and counted. The experiments were performed in triplicate.

2.6.2. Quantitative Reverse Transcription-PCR. RNA was extracted from clarified cell culture supernatants (16,000 g × 10 min) using PureLink Viral RNA/DNA Mini Kit (Invitrogen) according to the manufacturer’s instructions. RNA was eluted in 15 μL of RNase-free water and stored at −80 °C until use. Briefly, RNA was reverse-transcribed with SuperScript IV VILO (Invitrogen). 229E quantitation was performed by real-time PCR with an HCoV-229E-specific qPCR gene assay (Vi06439671_s1, Catalog number: 4,331,182, ThermoFisher). 23 SARS-CoV-2 quantitation was performed by real-time PCR with a PowerUp SYBR Green Master Mix (ThermoFisher) as follows: primers: RBD-qF1: 5′-CAATGCGTTTAACAGGCAAGG-3′ and RBD-qR1: 5′-CTCAAGGTGTCTGTGCAATCAGG-3′. A standard curve was generated by the determination of copy numbers derived from serial dilutions (10^3–10^8 copies) of the corresponding gene block (IDT Technologies). Each quantification was run in triplicate.

2.6.3. After-Effect Control. After-effect controls were conducted by mixing cell suspension with PSS-Bz-Ag, PSS-Bz, or PBS (10 s, see Section 2.6.1). The cells were then harvested and infected with viral inoculum for 1 h at 37 °C. The viral suspension was then incubated with susceptible cells for 1 h at 37 °C to allow for the viral particles to infect the cells. The virus inoculum was then removed, the cells were washed, and 1 mL of EMEM 2% FCS was added. The ability of the viral particles to infect susceptible cells was assessed on cell culture supernatants 24, 48, and 72 h postinfection (p.i.) by plaque assay. The experiments were performed in triplicate.

2.6.4. Interference Control. On the day of testing, sterile Petri dishes (35 mm diameter) were prepared, as previously reported (Section 2.3). Cell suspensions were added to the treated Petri dishes (PSS-Bz-Ag, PSS-Bz, or PBS (control)) for 1 h at room temperature, corresponding to the time period of the viral inoculum contact. The cells were then harvested and infected with a viral suspension (1.0 m.o.i.) for 1 h. The ability of the viral particles to infect susceptible cells was assessed on cell culture supernatants 24, 48, and 72 h postinfection (p.i.) by plaque assay. The experiments were performed in triplicate.

2.7. Statistical Analysis. Statistical comparisons of the data obtained from each treatment group were performed using Student’s t-test, as the data displayed a normal distribution based on the Kolmogorov–Smirnov test. Differences were considered significant at p < 0.05. Statistical analyses were performed using GraphPad Prism version 9.

3. RESULTS AND DISCUSSION

3.1. PSS-Bz-Ag and PSS-Bz Characterization. Elemental analysis and spectroscopic data for the silver complex were consistent with the presence of two MPA anionic ligands coordinated to Ag⁺ ions. The FT-IR spectrum of MPA showed the strong CO-stretching band of the carboxylic function at 1704 ± 2 cm⁻¹ and an S-H stretching band at 2555 ± 2 cm⁻¹; this band disappeared in the anionic silver complex, consistent with the coordination of sulfur to Ag⁺. Intense bands due to asymmetric and symmetric stretching modes of carboxylate appeared at 1580 ± 2 and 1386 ± 2 cm⁻¹, respectively.

The addition of benzalkonium chloride to the anionic silver complex (Ag(MPA))₂Cl⁻ in water, resulted in the neutralization of the negative charge (Bz)₃[Ag(MPA)]Cl. The salt could be solubilized in water by the addition of an excess of benzalkonium chloride to form micelles with diameters of the order of 2–3 nm and a zeta potential of ca. +9 mV. Subsequent addition of PSS to the micellar product allowed to obtain a polymeric material, PSS-Bz-Ag, insoluble in water, but exceedingly soluble in alcoholic solvents. Analogous polymers, PSS-Bz, were obtained by the addition of an excess of benzalkonium chloride to aqueous solutions of PSS. The two polymers did not show any appreciable water solubility, as shown by the analysis of the electronic absorption spectra of filtered suspensions and by the lack of the antimicrobial activity of the LB or Eagle MEM culture media left in contact for 20 h with the solid polymers (see solubility of the polymeric PSS-Bz-Ag and PSS-Bz components and Figure S1 and S2 in the Supporting Information).

The IR spectra of solid PSS-Bz-Ag and PSS-Bz (Figure S3) were similar, with vibrational bands matching the superposition of the intense bands of benzalkonium and PSS.

Solutions of PSS-Bz-Ag in alcohol were composed of micellar aggregates with diameters of the order of 10 nm and zeta potential of −3.9 mV (Figure S4a). Minority aggregates with higher diameters could be observed in the size distribution by intensity spectra (Figure S4b). Films of PSS-Bz-Ag and PSS-Bz polymers can be produced on any type of surface by spray coating or wiping the alcoholic solutions that evaporate quickly, leaving a very transparent layer, as shown in Figure 1.

![Figure 1. Glass slides untreated and treated with PSS-Bz-Ag and PSS-Bz.](https://doi.org/10.1021/acsami.1c10404)
PSS-Bz-Ag: (1 × 10^6 CFU) and PSS-Bz (1.2 × 10^6 CFU) - treated samples.

3.3. Cytotoxicity and Interference Tests for PSS-Bz-Ag and PSS-Bz Compounds. First, we assessed the cytotoxic effect of PSS-Bz-Ag or PSS-Bz toward human cells. We mixed...
the culture medium with PSS-Bz-Ag or PSS-Bz, and we used MRC5 and VeroE6 cell lines to perform the cytotoxic assay (MTT). We sprayed a plastic surface with PSS-Bz-Ag, PSS-Bz, or PBS and added the resulting solutions to MRC5 or VeroE6 cells for 5, 10, 15 min and 1, 24, 48, and 72 h. We observed that both PSS-Bz-Ag and PSS-Bz were not cytotoxic for human cells. In fact, the MTT assay (Figure 3a—d) and the viable cell count (Figure 3e—h) did not show any significant difference in comparison with PBS-treated cells (p: NS; Student’s t-test).

The interference control was performed by adding to cell cultures with PSS-Bz-Ag, PSS-Bz, or PBS for 1 h. PSS-Bz-Ag, PSS-Bz, or PBS solutions were removed, and the cells were infected with viral inoculum (1.0 m.o.i.) for 1 h. Viral titration showed no interference effect; in fact, viral plaques in PBS-treated samples did not differ from PSS-Bz-Ag- or PSS-Bz-treated samples (p: NS; Student’s t-test) (Table 1).

### 3.4. Antiviral Test for PSS-Bz-Ag and PSS-Bz Compounds

We tested the anti-229E and anti-SARS-CoV-2 effect of both PSS-Bz-Ag and PSS-Bz. The viral inoculum was exposed to a treated surface with PSS-Bz-Ag, PSS-Bz, or PBS. The time of contact of the viral inoculum was 5 min at room temperature, and then, the viral inoculum was recovered and used to infect MRC5 and VeroE6 cells and the target cells for 229E and SARS-CoV-2 in vitro assays. The viral inoculum was recovered after 1 h, and the cells were analyzed for viral titer 24, 48, and 72 h postinfection. These time points were selected as they allowed the detection of viral progeny formation, suggestive of viral infectivity. The viral load was determined by plaque assay. We observed a decreased viral plaque count after the treatment with both compounds in both cell lines, as reported in Table 2. At all time points, we observed a > 5 logarithmic reduction (LR) of plaque formation in 229E-infected samples treated with PSS-Bz-Ag or PSS-Bz (p < 0.001; Student’s t-test). SARS-CoV-2-infected samples showed a > 5 LR of plaque formation in the PSS-Bz-treated samples (p < 0.001; Student’s t-test). The PSS-Bz-Ag treatment was less effective at 24 h postinfection (p = 0.02; Student’s t-test) and reached a > 5 LR of plaque formation after 48–72 h postinfection (p < 0.001; Student’s t-test).

To obtain a further confirmation of the results obtained using the plaque formation test, we evaluated viral RNA by RT-qPCR in the culture medium collected from infected MRC5 and VeroE6 cells. We observed a decreased viral load after the treatment with both compounds in both cell lines (Table 3).

The results showed that PSS-Bz-Ag led to a 6.1 ± 0.6 LR for 229E RNA copies and a 3.7 ± 0.2 LR for SARS-CoV-2 RNA copies at 24 h postinfection (Table 3) (p < 0.001; p = 0.012, respectively; Student’s t-test). We observed a similar reduction of 229E RNA copies at different time points, while SARS-CoV-2 RNA copies were significantly reduced over time (24 vs 48 h p < 0.021; 24 vs 72 h p < 0.001; 48 vs 72 h p = 0.019; Student’s t-test). PSS-Bz led to a 6.5 ± 0.4 LR for 229E RNA copies and a 5.2 ± 0.4 LR for SARS-CoV-2 RNA copies at 24 h postinfection (Table 3). We observed a similar reduction of 229E and SARS-CoV-2 RNA copies over time (Table 3). These results confirm that both PSS-Bz and PSS-Bz-Ag are able to control 229E and SARS-CoV-2 infection of a target cell with a virucidal effect 24 h postinfection, according to the EMA guidelines, where a product is considered virucidal as soon as it has achieved a reduction of ≥4 logarithm.

To evaluate the possible after-effect of PSS-Bz and PSS-Bz-Ag, cell suspensions were added to the treated Petri dishes (PSS-Bz-Ag, PSS-Bz, or PBS (control)) for 1 h at room temperature, corresponding to the time period of the viral inoculum contact. The cells were then harvested and infected with a viral suspension (1.0 m.o.i.) for 1 h. The ability of the viral particles to infect susceptible cells was comparable to that of control samples (PBS-treated) at all time points (Table 4), demonstrating that both PSS-Bz and PSS-Bz-Ag are effective on viral particles and not on human cells.

Because both the main difference between PSS-Bz-Ag and PSS-Bz compounds is the presence of Ag in PSS-Bz-Ag, we evaluated the effect of the Ag complex alone at two concentrations, one corresponding to the concentration in PSS-Bz-Ag (0.0025% w/w) and a 50 times higher concentration (0.125% w/w) on 229E and SARS-CoV-2 infection. The Ag treatment had a slight effect on viral plaque formation, with both

### Table 1. Interference Test

| time postinfection (h) | logarithmic reduction (LR) (pfu/mL) |
|------------------------|-------------------------------------|
|                        | PSS-Bz-Ag   | PSS-Bz   |
| 229E                   | 0.3 ± 0.1   | 0.3 ± 0.1 |
| 48                     | 0.3 ± 0.1   | 0.2 ± 0.1 |
| 72                     | 0.2 ± 0.2   | 0.1 ± 0.2 |
| SARS-CoV-2             | 0.3 ± 0.1   | 0.3 ± 0.1 |
| 48                     | 0.2 ± 0.2   | 0.2 ± 0.1 |
| 72                     | 0.2 ± 0.1   | 0.1 ± 0.2 |

“LR of 229E and SARS-CoV-2 viral plaques after PSS-Bz-Ag, PSS-Bz, or Ag treatment in comparison with PBS-treated control. Data are reported as mean +/- standard deviation of three replicates.

### Table 2. LR of 229E and SARS-CoV-2 Viral Plaques after PSS-Bz-Ag, PSS-Bz, or Ag Treatment in Comparison with the PBS-Treated Control

| time post-infection (h) | LR (pfu/mL) |
|-------------------------|-------------|
|                         | PSS-Bz-Ag   | PSS-Bz   | Ag 0.0025% | Ag 0.108% |
| 229E                   | 5.9 ± 0.3   | 6.1 ± 0.5 | 0.2 ± 0.3 | 0.4 ± 0.3 |
| 48                     | 5.9 ± 0.8   | 5.9 ± 0.6 | 0.3 ± 0.4 | 0.4 ± 0.4 |
| 72h                    | 5.2 ± 0.6   | 5.9 ± 0.4 | 0.2 ± 0.3 | 0.3 ± 0.3 |
| SARS-CoV-2             | 3.8 ± 0.6   | 5.3 ± 0.5 | 0.9 ± 0.5 | 0.9 ± 0.4 |
| 48                     | 5.0 ± 0.3   | 5.5 ± 0.5 | 0.8 ± 0.6 | 0.8 ± 0.5 |
| 72                     | >7 ± 0.5    | 5.4 ± 0.4 | 0.8 ± 0.5 | 0.8 ± 0.5 |

“Data are reported as mean +/- standard deviation of three replicates.

### Table 3. LR of 229E and SARS-CoV-2 Viral RNA after PSS-Bz-Ag, PSS-Bz, or Ag Treatment

| time postinfection (h) | LR genome copies/mL |
|------------------------|----------------------|
|                        | PSS-Bz-Ag | PSS-Bz | Ag 0.0025% | Ag 0.108% |
| 229E                   | 6.1 ± 0.6 | 6.5 ± 0.4 | 0.5 ± 0.3 | 0.6 ± 0.4 |
| 48                     | 6.0 ± 0.3 | 6.4 ± 0.2 | 0.5 ± 0.2 | 0.6 ± 0.3 |
| 72h                    | 5.9 ± 0.4 | 6.3 ± 0.5 | 0.5 ± 0.2 | 0.6 ± 0.2 |
| SARS-CoV-2             | 3.7 ± 0.2 | 5.2 ± 0.4 | 0.8 ± 0.2 | 0.9 ± 0.3 |
| 48                     | 4.9 ± 0.5 | 5.3 ± 0.2 | 0.7 ± 0.2 | 0.8 ± 0.2 |
| 72                     | 6.8 ± 0.3 | 5.2 ± 0.1 | 0.6 ± 0.3 | 0.8 ± 0.3 |

“Data are reported as mean +/- standard deviation of three replicates.
Bz micellar assemblies, trapping the neutral [Ag(MPA)₂][Bz]₃ illustrated in Figure 4a,b.

Meanwhile, by evaluating the virucidal benzalkonium chloride demonstrated a reduction factor of >4 against SARS-CoV.24 Rabenau et al. found that both compounds are effective mainly on enveloped viruses as SARS-CoV-2, together with the alkyl chain “tail” component of benzalkonium chloride, that perturbs the membrane bilayer by permeating the barrier and disrupting its physical and biochemical properties. Protein function is subsequently disturbed, and the combination of these effects results in the solubilization of the bilayer constituents into benzalkonium chloride/phospholipid micelles.

Table 4. After-Effect Test

| time postinfection (h) | PSS-Bz-Ag (LR (pfu/mL)) | PSS-Bz (LR (pfu/mL)) |
|------------------------|-------------------------|----------------------|
| 24                     | 0.2 ± 0.1               | 0.1 ± 0.1            |
| 48                     | 0.1 ± 0.2               | 0.1 ± 0.1            |
| 72                     | 0.1 ± 0.2               | 0.1 ± 0.1            |

“Logarithmic reduction of 229E and SARS-CoV-2 viral plaques after PSS-Bz-Ag, PSS-Bz, or Ag treatment in comparison with the PBS-treated control. Data are reported as mean ± standard deviation of three replicates.

Ag concentrations (Table 2). Interestingly, the effect is not concentration- and time-dependent (Table 2). RNA copy analysis confirmed the plaque assay results (Table 3), showing a slight LR of RNA copies with both Ag concentrations. These results support the hypothesis that the main virucidal component is benzenonium chloride. Literature data support this efficacy based on the cationic “headgroup” of benzalkonium chloride that is progressively adsorbed to the negatively charged phosphate heads of phospholipids in the lipid bilayer. This might be effective mainly on enveloped viruses as SARS-CoV-2, together with the alkyl chain “tail” component of benzalkonium chloride, that perturbs the membrane bilayer by permeating the barrier and disrupting its physical and biochemical properties. Protein function is subsequently disturbed, and the combination of these effects results in the solubilization of the bilayer constituents into benzalkonium chloride/phospholipid micelles.

Multiple studies have reported on the virucidal effect of benzalkonium chloride against coronaviruses. Rabenau et al. found that, as a surface disinfectant in a concentration of 0.5%, benzalkonium chloride demonstrated a reduction factor of >4 against SARS-CoV-2.24 Meanwhile, by evaluating the virucidal activity of different oral rinses against three strains of SARS-CoV-2, Meister et al. reported log reductions of >3.1, >2.8, and >2.6, respectively, for a rinse containing 0.035% benzalkonium chloride that is progressively adsorbed to the negatively charged phosphate heads of phospholipids in the lipid bilayer. This might interfere with SARS-CoV-2 replication, with a time-dependent effect. The strong efficacy of both compounds on 229E infection supports the different resistances of these two coronaviruses.26 The results obtained with interference and after-effect tests confirm that both compounds are effective during the 5 min of contact, and they are not removed within the viral inoculum. The water insolubility of these products should allow and help maintain their effectiveness over time.

4. CONCLUSIONS

We have examined the antibacterial and antiviral properties of polymeric compounds that can be easily synthesized by coupling positively charged antimicrobial agents (either as a cationic molecule or metal complex) with a negatively charged polymer such as sulfonated polystyrene. Our general approach has been to produce a water-insoluble material, which could be solubilized in a volatile organic solvent for spray-coating surfaces. Specifically, we have analyzed the properties of the transparent polymeric formulations consisting of polystyrene sulfonate, silver, and benzalkonium or simply by polystyrene sulfonate and benzalkonium. These studies support a very high antibacterial and antiviral effect toward 229E and SARS-CoV-2, as evidenced by a > 5 LR in the infectious agent after 5 min of contact with the treated surface. The reported examples can be useful for the production of new polymeric materials containing different cationic antimicrobial species.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsami.1c10404.

ASSOCIATED CONTENT

Solubility of the polymeric PSS-Bz-Ag and PSS-Bz components, FT-IR spectra, and the size distribution of PSS-Bz-Ag (PDF).

AUTHOR INFORMATION

Corresponding Authors

Carlo Alberto Bignozzi – Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy; Phone: 00390532455130; Email: g4s@unife.it

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Robert Rizzo — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy; Phone: 00390532453382; Email: rbr@unife.it; Fax: 00390532974470

Valentina Gentili — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy

Daniele Pazzi — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy

Sabrina Rizzo — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy

Giovanna Schiuma — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy

Edoardo Marchini — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy; orcid.org/0000-0002-8092-1349

Stefania Papadia — Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Ferrara 44100, Italy

Andrea Sartorel — Department of Chemical Sciences, University of Padova, Padova 35131, Italy; orcid.org/0000-0002-4310-3507

Dario Di Luca — Department of Medical Sciences, University of Ferrara, Ferrara 44100, Italy

Francesca Caccuri — Department of Microbiology and Virology, Spedali Civili, Brescia 25125, Italy

Complete contact information is available at: https://pubs.acs.org/10.1021/acsami.1c10404

Author Contributions

R.R., C.B.: conceptualization; data curation; funding acquisition; V.G.: investigation; methodology; project administration; D.P.: antibacterial analysis; E.M., S.P.: chemical compound synthesis; A.S.: dimensional analysis and Z potential quantification; S.R., G.S., F.C., D.D.L.: antiviral analysis. V.G.: investigation; methodology; project administration; A.S.: dimensional analysis and Z potential quantification; S.R., G.S., F.C., D.D.L.: antiviral analysis.

Notes

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ABBREVIATIONS

LR, logarithmic reduction
QA, quaternary ammonium
KBr, potassium bromide
Bz, benzalkonium
CFU, colony-forming units
TSA, tryptone soya agar
MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide

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