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Development of Environmentally Friendly Biocidal Coatings Based on Water-soluble Copolymers for Air-cleaning Filters

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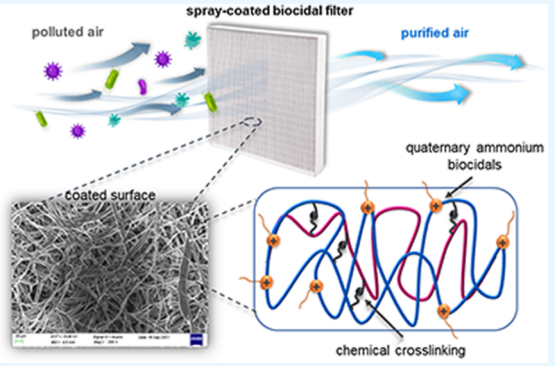
ABSTRACT: Air pollution by pathogens has posed serious concern on global health during the last decades, especially since the breakout of the last pandemic. Therefore, advanced high-efficiency techniques for air purification are highly on demand. However, in air-filtering devices, the prevention of secondary pollution that may occur on the filters remains a challenge. Toward this goal, in the present work, we demonstrate a facile and eco-friendly process for the biocidal treatment of commercial high-efficiency particulate air filters. The antibacterial filters were successfully prepared through spray coating of aqueous solutions based on biocidal water-soluble polymers, poly(sodium 4-styrene sulfonate-*co*-cetyl trimethylammonium 4-styrene sulfonate-*co*-glycidyl methacrylate) [P(SSNa24-*co*-SSAmC$_{16}$56-*co*-GMA20)] and poly(2-dimethylaminoethyl)methacrylate. Significantly, an optimized green route was developed for the synthesis of the used polymers in aqueous conditions and their stabilization through cross-linking reaction, leading to biocidal air filters with long-lasting activity. The developed coatings presented strong and rapid antibacterial activity against *Staphylococcus aureus* (in 5 min) and *Escherichia coli* (in 15 min). Moreover, the cytotoxicity test of the polymeric materials toward A549 lung adenocarcinoma cells indicated very low toxicity as they did not affect either the cell growth or cell morphology. The above-mentioned results together with the scalable and easy-to-produce green methodology suggest that these materials can be promising candidates as filter coatings for use on air-purification devices.



1. INTRODUCTION

The spread of airborne diseases such as the last pandemic has demonstrated the urgent need for new strategies capable of purifying the air, with enhanced antimicrobial and antiviral activity. Some of the most explored technologies consist of ultraviolet (UV)-C light or plasma air ionization devices as well as filter-based protective equipment. Although commercial filters such as high-efficiency particulate air filters (HEPA) can effectively block the passage of submicron-sized particulates,[1] it has been shown that microorganisms may accumulate on the filters, which can become a secondary polluting source.[2] Therefore, development of efficient air filters with inherent antimicrobial activity is currently of utmost importance.

Among the different approaches, modification of existing filter technologies with broad-range biocides such as metal ions, biopolymers, and carbon-based nanomaterials has been widely investigated.[3−7] The most widely used antimicrobial is silver, which has been incorporated in or coated on filters, in a wide range of forms.[8−12] Copper nanoparticles have also been extensively studied as antimicrobial agents on air filters,[13] in the form of copper oxide (CuO)[14,15] or in the form of copper sulfate.[16,17]

Additionally, some biopolymers or low-molecular-weight biocides have been used as attractive alternatives for the fight against bacteria and viruses.[18−20] For instance, modification of filters with chitosan,[21] alginate,[22] gelatin,[23,24] and herbal extracts[25−27] has been reported to perform efficiently in air-purification systems. In a recent study, HEPA filters modified with tannic acid demonstrated ∼90% capture of the H^{1}N^1 virus compared to the neat filters.[28] In another work, filters modified with the broad-range biocide chlorhexidine digluconate could kill pathogens in under 15 min and destroy SARS-CoV-2 viral particles in under 30 s.[29]

Although the above-mentioned strategies for modification of the air filters have shown promising results on bacterial inhibition or viral inactivation, they may present some thoughtful disadvantages, such as multi-step production, cytotoxicity, or lack of stability. More specifically, the release

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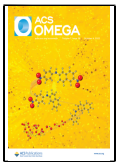

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of nanoparticles (e.g., CNTs and metal NPs) into air may cause adverse effects on human health.[30,31] Thus, additional attempts are focusing on the development of cost-effective and environmentally friendly coating materials based on polymeric biocidal compounds, taking advantage of their low toxicity and zero migration or leaching properties providing high efficiency and stability on filter surfaces.[26,32−35]

Biocidal coatings based on covalently attached and/or electrostatically bound, quaternized ammonium functionalities have been recently developed by our group, through the combination of adequate polymeric structures of both active biocidal species and reactive functionalities that can stabilize the water-based coatings after reaction in the solid state.[36−39] However, the design of these materials involved one or more reaction steps in organic solvents. In the present work, toward a greener direction, a major novelty is the establishment of water-based processes for all steps involved, from the synthesis of polymer precursors up to the final coating. Specifically, poly(sodium 4-styrene sulfonate-*co*-glycidyl methacrylate), P(SSNa-*co*-GMA*x*), copolymers have been prepared in water and used for the electrostatic binding of quaternized hexadecylammonium groups (AmC_{16}) at the desired molar ratio to maintain water solubility. Moreover, the water-soluble poly(2-dimethylaminoethyl methacrylate), PDMAEMA homopolymer, known for its inherent antibacterial properties,[40] has been used as the cross-linking agent. Selected blends of these polymers in aqueous solution were then used for treatment of polypropylene HEPA filters under optimized conditions. Furthermore, the air-flow test revealed no effect of the polymeric coating on the air permeability, while examination of the coated filters' surface showed successful and homogeneous modification. To verify the biocidal efficiency and safety of the final setup, antibacterial and cytotoxicity tests were conducted on the individual polymers as well as on the polymeric coatings. The results showed rapid killing efficiency against bacterial species and no health risk was revealed by the in vitro cytotoxicity tests of the polymeric materials.

2. EXPERIMENTAL SECTION

2.1. Materials. The monomers glycidyl methacrylate (GMA), sodium 4-styrene sulfonate (SSNa), and (2-dimethylaminoethyl)methacrylate (DMAEMA), the surfactant cetyltrimethylammonium bromide (CTAB), the initiators potassium persulfate ($K_2S_2O_8$) and potassium metabisulfite ($K_2S_2O_5$), as well as deuterium oxide (D_2O) and deuterated dimethyl sulfoxide (DMSO-d_6) were purchased from Aldrich and used as received. Ultra-pure water was obtained by means of an SG apparatus water purification unit. The copolymers P(SSNa-*co*-GMA30) and P(SSNa-*co*-GMA40), containing 30 and 40% mol GMA, were synthesized as reported previously.[41,42] The homopolymers poly(sodium 4-styrene sulfonate), PSSNa, and poly(glycidyl methacrylate), PGMA, were previously synthesized by our group.

2.2. Synthesis of the Polymeric Materials. *2.2.1. Synthesis of the Copolymer P(SSNa-co-GMA20) through Aqueous Free Radical Polymerization.* The copolymer poly(sodium 4-styrenesulfonate-*co*-glycidyl methacrylate) was synthesized through free radical polymerization using a redox initiator in aqueous media at 40 °C. This copolymer is denoted as P(SSNa-*co*-GMA20), where 20 is the mol percentage of GMA units in the copolymer, as determined by the ^{1}H NMR characterization in DMSO-d_6. Briefly, the monomer SSNa was dissolved in degassed distilled water (15% w/v), and then, GMA was added to the solution and left under vigorous stirring at room temperature until a clear homogeneous solution was obtained. Afterward, the pair of initiators $K_2S_2O_8$/$K_2S_2O_5$ (1% mol of each over the total monomers' concentration) was added and the reaction was left to proceed for 18 h under vigorous stirring in an Ar atmosphere in an oil bath set at 40 °C. After cooling down to room temperature, the reaction mixture was precipitated in acetone, washed with acetone, and further dried at 40 °C for 48 h. The P(SSNa-*co*-GMA20) copolymer was characterized by ^{1}H NMR and attenuated total reflection Fourier transform infrared spectroscopy (ATR−FTIR) spectroscopy, and its molecular weight was determined through size exclusion chromatography (SEC).

2.2.2. Synthesis of PDMAEMA Homopolymer. The PDMAEMA homopolymer was synthesized via free radical polymerization using a redox initiator in aqueous media as follows: In a round bottom flask, the monomer DMAEMA was first dissolved in water (15% w/v), and then, the initiators $K_2S_2O_8$/$K_2S_2O_5$ (1% mol over the total monomer's concentration) were added to the solution. The reaction mixture was stirred at 35 °C for 48 h under an argon atmosphere, and then, it was dialyzed against water and freeze-dried. The obtained polymer was characterized by ^{1}H NMR and ATR−FTIR spectroscopy, whereas the molecular weight was determined by intrinsic viscosity measurements.

2.2.3. Introduction of Biocidal Units to P(SSNa-co-GMAx) Copolymers. For the introduction of the biocidal units in the P(SSNa-*co*-GMA*x*) copolymers, an ion exchange reaction in aqueous solution between the sodium ions of SSNa units with the quaternary cetyltrimethylammonium cations (AmC_{16}) of CTAB was carried out. A typical procedure consists of pouring the CTAB aqueous solution (10% w/v) dropwise into the aqueous solution of the copolymer P(SSNa-*co*-GMA*x*) (5% w/v) under vigorous stirring at room temperature. To find the optimal conditions that would provide a final water-soluble copolymer, several parameters were investigated, as detailed below.

2.2.4. Preparation of the Biocidal Filters. Air filters (meltblown PP, H13 class) were uniformly coated with the polymeric solution using a spray gun. The coating process was carried out on a clean bench, and afterward, the filter material was dried at room temperature for 48 h. The polymeric loading on the filters was calculated by the equation: (% w/w) = $(W - W_0)/W_0$%, where w is the weight of the coated filter after drying and w_0 is the weight of the uncoated filter.

2.3. Characterization Techniques. The polymers P(SSNa-*co*-GMA*x*), P(SSN*a*x-*co*-SSAmC_{16}*y*-*co*-GMA*z*), and PDMAEMA were characterized by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) using a Bruker AVANCE DPX 400 spectrometer, at 400 MHz, 300 K and were dissolved in DMSO-d_6 or D_2O. ATR−FTIR was also conducted for the characterization of the polymers and the coating using a Bruker Platinum ATR−FTIR spectrometer. For the determination of the water-soluble P(SSNa-*co*-GMA*x*) precursors' molecular weight, SEC was performed using a Millipore Waters 501 HPLC chromatographer at 25 °C, equipped with two Shodex B-804, B-805 linear columns (8 mm × 500 mm), a differential refractometer (R401) detector, poly(ethylene oxide) standards, and 0.1 M $LiNO_3$ as the eluent. The operating flow rate was set at 1 mL/min. For the determination of the molecular weight of PDMAEMA, the polymer was dissolved in tetrahydrofuran at various concentrations up to 0.25% w/v and the intrinsic viscosity at 35 °C was determined using an

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Ostwald viscometer. The molecular weight was calculated using the Mark−Houwink−Sakurada equation: $[\eta] = KM^{\alpha}$, where the values of K and α are 4.98×10^{-3} ($cm^3\ g^{-1}$) and 0.729, respectively, as reported in the literature.[43]

The coated filters' surface morphology was investigated by scanning electron microscopy (SEM, Zeiss SUPRA 35VP instrument), whereas energy-dispersive X-ray spectroscopy (EDS) was also performed for the elemental analysis of the polymeric coating. The filter's modification with the polymeric materials was further investigated by water contact angle measurements. Specifically, a 10 μL droplet of ultra-pure water was pipetted onto the surfaces of the coated and uncoated filters and contact angles were measured using ImageJ software. Finally, an air-flow rate test was carried out to examine the effect of polymeric coating on air permeability through the filter. For this purpose, uncoated HEPA filters and coated filters with the polymeric mixture (loading 8%) were put in a filter holder and installed in an industrial condensing air device. The air-flow rate was measured by a Digital Anemometer (HP-836A) on three different sides of the device. For each measurement, the anemometer probe was held until stability of the reading.

2.4. Physicochemical Characterization. *2.4.1. Turbidity Studies.* The optical density at 500 nm was determined using a HITACHI U-1800 UV−Vis spectrophotometer equipped with a circulating water bath, set at 25 °C. The concentration of the polymeric aqueous solutions was fixed at 3% w/v.

2.4.2. Rheological Measurements. The shear viscosity and frequency sweep tests were performed at 25 °C using a Discovery Hybrid Rheometer 2.0 (TA Instruments, DE, United States) equipped with cone-plate geometry (diameter 40 mm, gap 0.055 mm). All samples were left for at least 1 min in the apparatus to equilibrate before measurement. Preliminary tests were performed to define the linear viscoelastic region.

2.5. Antibacterial Activity Test. First, cultures of the studied bacterial species were prepared. Hence, *Escherichia coli* MC1061 and 9001, *Pseudomonas aeruginosa* NCTC 10662, and *Staphylococcus aureus* NCTC 6571 strains were used as representatives of Gram-negative and Gram-positive microorganisms to test the antibacterial activity of the polymers and their respective blends. MC1061 was from our lab collection, whereas other strains were from the Health Protection Agency, Porton Down, Salisbury, UK. Single colonies of each strain were cultured in 8 mL of LB broth overnight (18 h) in 15 mL tubes laying flat at 80 rpm, 37 °C, to a final cell density of approximately 10^8 to 10^9 cfu mL^{-1}

A time-kill assay was conducted for the antimicrobial efficiency testing of the synthesized polymeric materials. Precisely, glass coupons (18 × 18 mm) were coated with the polymeric solutions under sterile conditions and left to dry at RT overnight. 20 μL aliquots of overnight (18 h) cultures of each bacterial species were placed on the coated glass coupons and incubated at 22 °C for different time intervals (5, 15, 30, 60, and 120 min). Next, the coupons were dipped four times in a LB medium (30 mL LB in 50 mL tubes), and the cultures were placed horizontally and incubated at 80 rpm, at 37 °C, for 5 h in the case of *E. coli*, 7 h for *P. aeruginosa,* and 16 h for *S. aureus*. Cell growth (scattering) was measured at 600 nm, diluting if needed the samples in water so that the final A_{600}s were ≤0.5. The volume of inoculation (20 μL) and the time of growth for each species ensured that all measurements were performed when control cultures (no polymer exposure) were at their exponential phase of growth (growth curves at Figure S1). The time-kill assays were performed in triplicates on different days with bacteria from different starting cultures. The effect of the polymers on cell viability was estimated by comparing the growth of the controls to that of cells exposed to polymers by the following equation: $\text{cellviability\%} = \frac{OD_{600}\text{sample}}{OD_{600}\text{control}} \times 100\%$.

In the bar graphs presenting bacterial toxicities, all mean values are the means of two different experiments each performed in triplicates. Error bars represent the standard error for the two means.

2.6. In Vitro Cytotoxicity Study. Human lung adenocarcinoma A549 cell line was obtained from the American Type Culture Collection (ATCC, Baltimore, MD, USA) and routinely cultured as monolayers at 37 °C in a humidified atmosphere of 5% (v/v) CO_2 and 95% air. Cells were cultured in complete DMEM culture medium supplemented with 10% fetal bovine serum (FBS), a cocktail of antimicrobial agents (100 IU/mL penicillin, 100 mg/mL streptomycin, 10 mg/mL gentamicin sulfate and 2.5 mg/mL amphotericin B), 2 mM L-glutamine, and 1 mM sodium pyruvate. Cells were harvested by trypsinization with 0.05% (w/v) trypsin in PBS containing 0.02% (w/v) Na_2EDTA. All experiments were conducted in serum-free conditions (0% FBS). PDMAEMA, P(SSNa24-*co*-SSAmC_{16}-*co*-GMA20), and the mixture 90/10% (w/w) of them were dissolved in H_2O. The concentration range of each polymer used in this study was 1−50 with 10 μg/mL increments (the low and high concentrations are shown).

2.6.1. Crystal Violet Cell Viability Assay. 48-well cell culture plates were coated with each polymer for 24 h at room temperature, and A549 cells were seeded on top of the coatings at DMEM 2% FBS and incubated for 24 h. After the incubation period, cells were stained with 0.5% (w/v) crystal violet solution in 20% methanol/distilled water for 20 min at 37 °C with 150 oscillations on a bench rocker. The stained cells were left to dry at room temperature for 24 h. Methanol was added to each well, and the cell-bound dye was retrieved after 20 min incubation of the plate at 150 oscillations on a bench rocker. Following the incubation, the optical density of each well was measured at 570 nm using a TECAN photometer, utilizing Magellan 6. Photographs were captured utilizing a color digital camera (CMOS) on a phase contrast microscope (OLYMPUS CKX41, QImaging Micro Publisher 3.3RTV) through 10× and 40× objective to monitor cell morphology.

2.6.2. MTT Cell Viability Assay. A549 cells had been seeded in 96-well cell culture plates and were grown up to 60−70% confluency, followed by a 16 h starvation in a serum-free medium, prior to treatment in the presence or absence of the polymer in the concentration range of 1−50 with 10 μg/mL increments. To evaluate the biopolymer effects on cell viability through mitochondrial dehydrogenase activity, MTT (water-soluble tetrazolium salt) was added to each well at a ratio of 1:10, and the absorbance at 450 nm was measured (reference wavelength at 650 nm).

3. RESULTS AND DISCUSSION

The main goal of the present work was the preparation of environmentally friendly materials with antibacterial activity for their use as stable coatings on air-cleaning filters. Biocidal treatment of HEPA filters was performed by spray coating of an aqueous polymeric solution P(SSNa*x*-*co*-SSAmC_{16}*y*-*co*-

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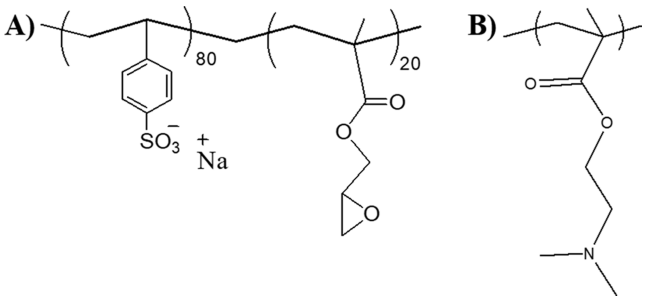
Scheme 1. Structures of the Synthesized Copolymer P(SSNa-*co*-GMA20) (A) and Homopolymer PDMAEMA (B)



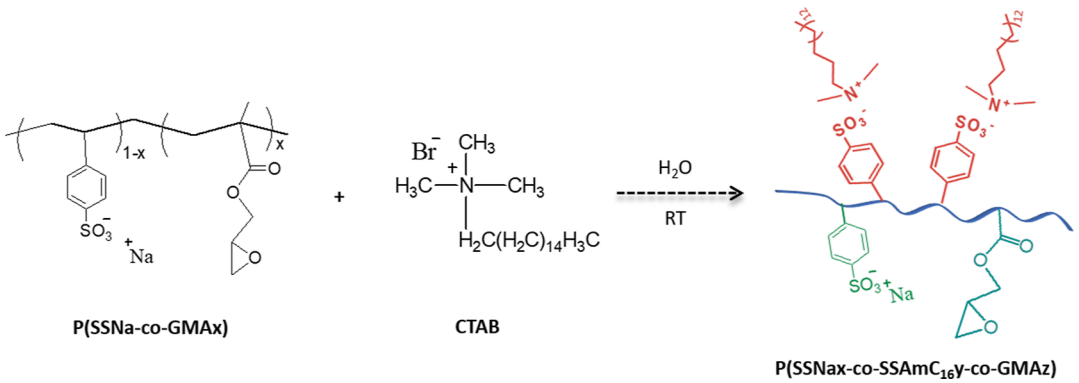
GMA*z*)/PDMAEMA. First, a thorough investigation was made on the optimization of the polymerization procedures providing water-soluble antimicrobial polymers. The next step was the study on the cross-linking reaction of the polymeric mixture P(SSN*a*-*co*-SSAmC$_{16}$*y*-*co*-GMA*z*)/PDMAEMA, which was an essential step for creating a stable polymeric coating with long-lasting activity. Furthermore, examination of the coated filters revealed successful and homogeneous modification of the surface, which does not affect the air permeability through the filter. Finally, a systematic study of the biocidal activity and cytotoxicity tests were performed supporting the high activity and low toxicity of the studied materials.

3.1. Water-based Synthesis of Polymeric Precursors. Toward the development of an environmentally benign methodology for the production of biocidal air filters, a crucial parameter of this work was the preparation of the polymeric materials under mild aqueous conditions. Herein, we report the synthesis of the polymeric precursors P(SSNa-*co*-GMA20) and PDMAEMA using a typical free radical polymerization procedure in an aqueous medium (Scheme 1). It is worth mentioning that the P(SSNa-*co*-GMA*x*) copolymer has already been synthesized by our group in organic solvents (DMSO or DMF)[30,31] since the solubility of GMA in water is limited. To the best of our knowledge, surfactant-free copolymerization of SSNa with GMA in aqueous medium has been reported once before in the literature, with a monomer molar ratio of 3:2, using ammonium persulfate (APS) as the initiator at 75 °C, resulting in gelation of the reaction mixture due to the ring opening of the epoxide group of GMA.[44] To avoid gelation, in the present study, we performed the copolymerization at 35−40 °C using the redox initiator couple $K_2S_2O_8/K_2S_2O_5$ since it functions successfully at low temperatures. The polymerization mixture appeared highly viscous after 6 h, but most importantly, the resultant copolymer product was soluble in water. Synthesis of the copolymer was verified by ATR−FTIR spectroscopy and ^{1}H NMR spectroscopy in DMSO-d_6 (Figure S2A,B). The GMA content of the copolymer was calculated from the ^{1}H NMR spectrum as 20 mol % (details in Supporting Information), while the molecular weight (M_n: 80,000, M_w: 185,000, PDI: 2.3) was determined through SEC in aqueous 0.1 M $LiNO_3$ solution using poly(ethylene oxide) standards.

The same procedure was followed for the aqueous polymerization of PDMAEMA using the redox initiator couple $K_2S_2O_8/K_2S_2O_5$, at a fixed temperature of 35 °C, since the polymer shows a thermo-responsive behavior and separates out from water at slightly higher temperatures (the low critical solution temperature (LCST) is ∼40 °C).[40] As far as we are aware, the polymerization of DMAEMA in aqueous medium is rarely reported, mostly using controlled polymerization techniques (RAFT and ATRP).[45,46] The synthesis by aqueous free radical polymerization at 60 °C using APS as the initiator has also been reported in the past.[47] The structure of the polymer product was verified through ^{1}H NMR spectroscopy in D_2O (Figure S3). The Ostwald viscometer method was used for the determination of the homopolymer's molecular weight, as described in the experimental section (2.4.2). Intrinsic viscosity measurements of PDMAEMA solutions in THF, at 35 °C, provided a high value of molecular weight (M_w: 100,000).

3.2. Introduction of Biocidal Groups into the P(SSNa-*co*-GMA*x*) Copolymers. The second important goal toward an exclusively aqueous process was the preparation of water-soluble antibacterial polymeric materials from aqueous solutions. Toward this direction, a thorough investigation was performed to ion exchange the Na^+ cations of the water-soluble P(SSNa-*co*-GMA*x*) copolymers with the cetylammonium cations (AmC_{16}) of the broad-range biocidal quaternary ammonium salt, CTAB, while maintaining water solubility. In fact, the introduction of AmC_{16} cations in P(SSNa-*co*-GMA*x*) copolymers close to CTAB/SSNa stoichiometry has been reported in previous studies,[26,27] leading to water-insoluble P(SSAmC$_{16}$-*co*-GMA*x*) copolymers. Therefore, in the present work, we focus on the synthesis of water-soluble P(SSN*a*-*co*-SSAmC$_{16}$*y*-*co*-GMA*z*) terpolymers through partial exchange of the sodium cations of SSNa units with the quaternary cetylammonium cations (AmC_{16}) of CTAB in aqueous solution (Scheme 2).

Scheme 2. Reaction Route for the Synthesis of the Water-soluble Terpolymer P(SSN*a*-*co*-SSAmC$_{16}$*y*-*co*-GMA*z*)



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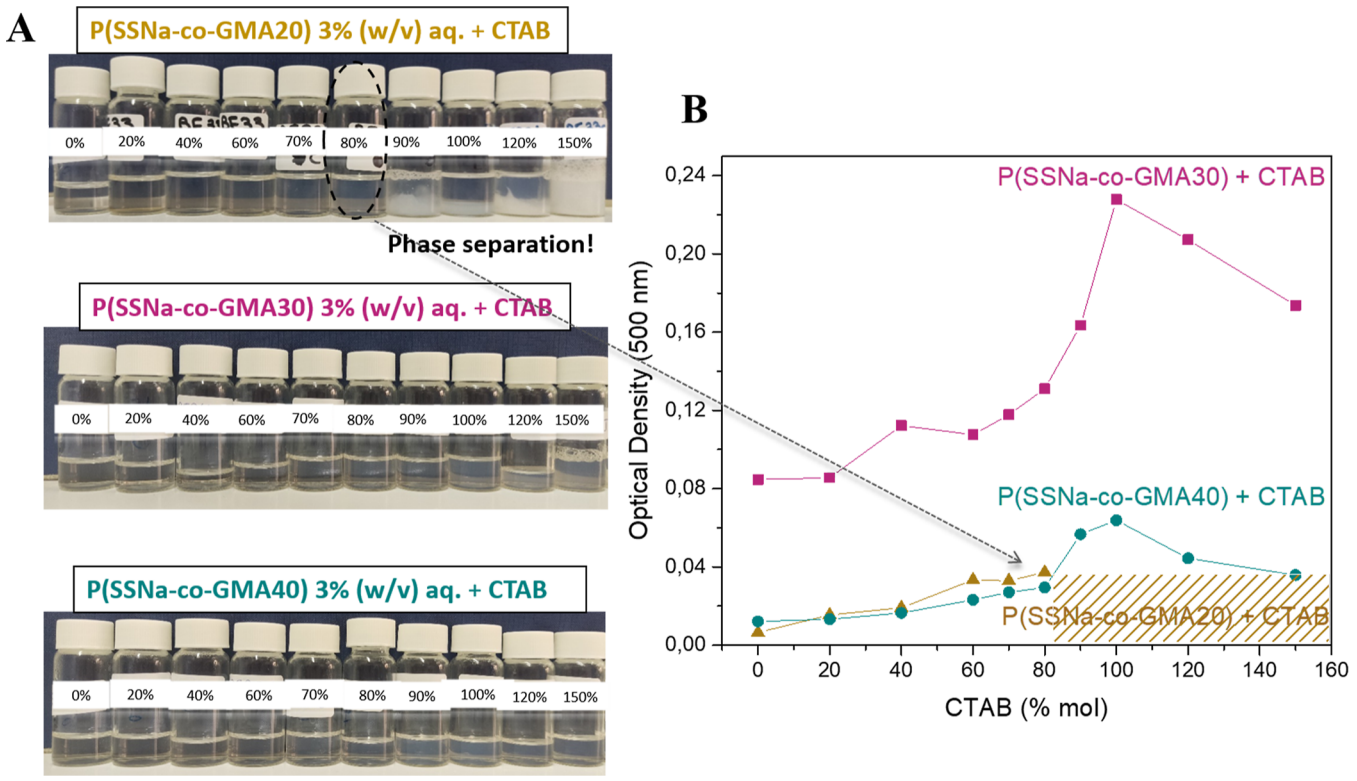

Figure 1. Optical observation (A) and turbidity measurements (B) of the aqueous mixtures P(SSNa-*co*-GMA*x*)/CTAB in various molar mixing ratios of CTAB/SSNa (0−150%).

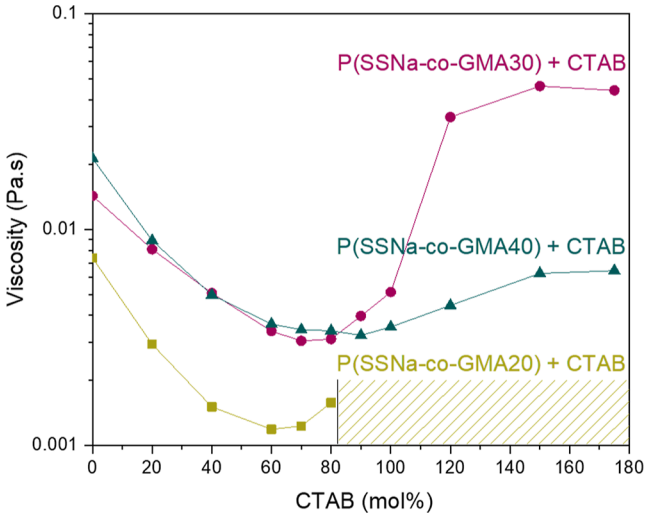

Figure 2. Viscosity measurements of the polymeric solutions of P(SSNa-*co*-GMA*x*), where *x*: molar ratio of GMA (20, 30, and 40%), after the introduction of CTAB in different molar ratios (20−175%). The measurements were taken by rheometer (A,B) and Ostwald viscometer (C).

In this detailed study, the behavior of aqueous mixtures of CTAB with three water-soluble P(SSNa-*co*-GMA*x*) copolymers with different % mol GMA contents (*x* = 20, 30, and 40%) was investigated. The polymer concentration was fixed at 3% w/v, while the % molar ratio (*r*) of CTAB/SSNa units covered the range *r* = 0−150%. Optical observation and turbidity measurements of the polymeric solutions (Figure 1A,B) showed that the behavior depends on both GMA content and molar ratio *r*. More specifically, in the case of P(SSNa-*co*-GMA20) copolymer, when r exceeds 70%, macroscopic phase separation takes place, as it is usually observed when polyelectrolytes like poly(sodium styrene sulfonate), PSSNa, form mixed associates with oppositely charged surfactants.[48,49] This is also observed when CTAB interacts with copolymers of sodium styrene sulfonate with hydrophobic monomers like styrene, (P(SSNa-*co*-St), or methyl methacrylate (P(SSNa-*co*-MMA), with hydrophobic contents comparable to those of our copolymer.[50,51] Moreover, in qualitative agreement with the behavior found for P(SSNa-*co*-MMA) copolymers, macroscopic phase separation does not take place and turbidity is practically suppressed or a marginal turbidity increase is observed when *r* > 80%, for the copolymers with higher GMA contents, *x* = 30 or 40%.

Regarding the application of the final product, among the most critical characteristics of the polymeric solution is its viscosity. A low viscosity of the solution is desired since it is intended to be loaded on air filters by the spraying technique using a spray gun. Thus, the viscosity of the aqueous mixtures was followed as a function of *r*. In the case of the system P(SSNa-*co*-GMA20)/CTAB, an Ostwald viscometer was used because the viscosity was too low (Figure 2C), whereas the other systems were measured by a rheometer (Figure 2A,B). As it is seen, addition of CTAB in the P(SSNa-*co*-GMA*x*) solutions initially leads to a significant viscosity decrease, as usually expected for the association of surfactants with oppositely charged polyelectrolytes.[52] As seen in Figure 2A,B, the further addition of CTAB leads to a viscosity enhancement, especially in the case of P(SSNa-*co*-GMA30). Such a viscosity enhancement, much more pronounced however, has also been observed in the case of (PSSNa-*co*-MMA) copolymers for similar r values, and it was attributed to the possible formation of hybrid wormlike micelles.[50]

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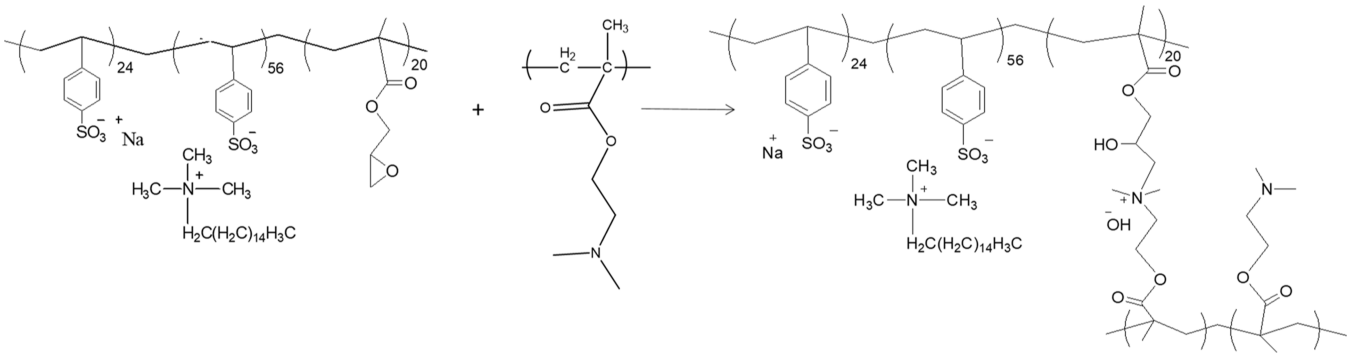
Scheme 3. Proposed Reaction Mechanism between the Polymers P(SSNa24-*co*-SSAmC$_{16}$56-*co*-GMA20) and PDMAEMA in Aqueous Conditions



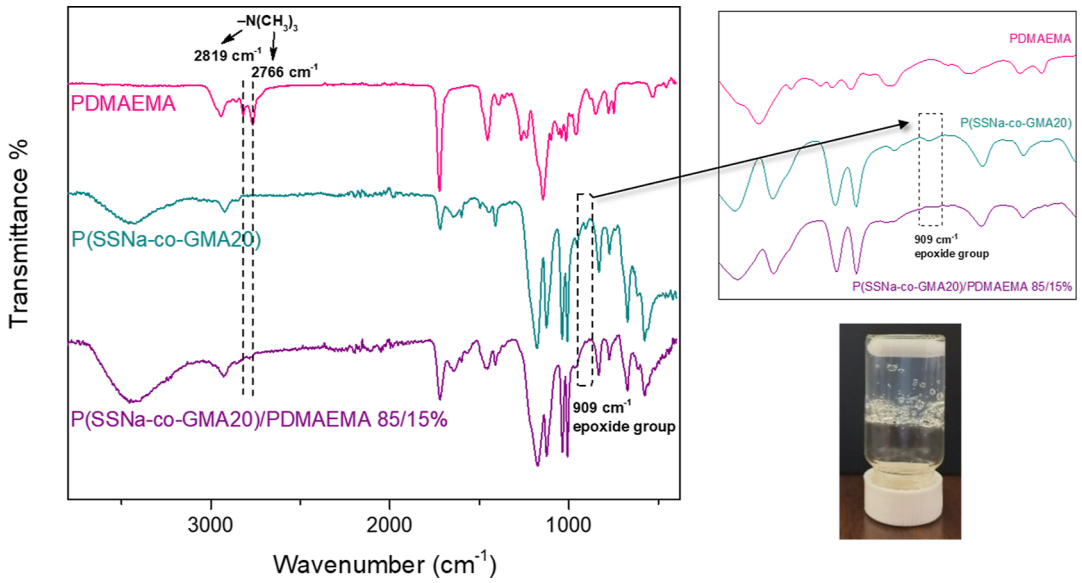


Figure 3. ATR−FTIR spectrum of the cross-linked gel formed by the polymeric mixture P(SSNa-*co*-GMA20)/PDMAEMA 85/15%, after drying at room temperature. Spectra of the initial polymers P(SSNa-*co*-GMA20) and PDMAEMA are shown as well for comparison.

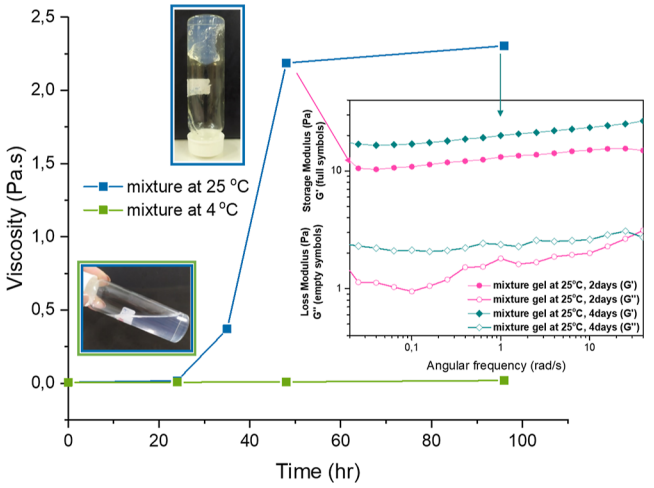


Figure 4. Rheology studies of the polymeric mixtures P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w). Investigation on the effect of the parameters time and temperature (4 and 25 °C) on the cross-linking reaction. The gelation process is shown in the photographs for the study at 25 °C. The total polymer concentration is 6% w/w.

Concerning the present targeted application, Figure 2 shows that the lower values of viscosity are observed for the P(SSNa-*co*-GMA20)/CTAB solution just before phase separation. Based on these results, we decided that the most suitable system to proceed was the copolymer P(SSNa-*co*-GMA20) with 70%mol CTAB. According to the characterization by ^{1}H NMR spectroscopy (Figure S4), the calculated molar ratio of CTAB is in good agreement with the feed composition; thus, this system will be denoted as P(SSNa24-*co*-SSAmC$_{16}$56-*co*-GMA20).

3.3. Cross-linking Studies. After the successful synthesis and characterization of the water-soluble terpolymer P-(SSNa24-*co*-SSAmC$_{16}$56-*co*-GMA20) and the homopolymer PDMAEMA, the next step was to develop antibacterial polymeric coatings derived from their aqueous polymeric mixtures. The homopolymer PDMAEMA was especially chosen for this work because it combines two significant properties: the inherent efficacy to inhibit bacterial growth[40] and the reactivity of the tertiary amine group. Specifically, as known,[53,54] the amine group can attack the oxirane ring of GMA of the terpolymer P(SSNa24-*co*-SSAmC$_{16}$56-*co*-GMA20) via nucleophilic addition reaction leading to a cross-linked polymeric network as shown in the proposed mechanism in Scheme 3.

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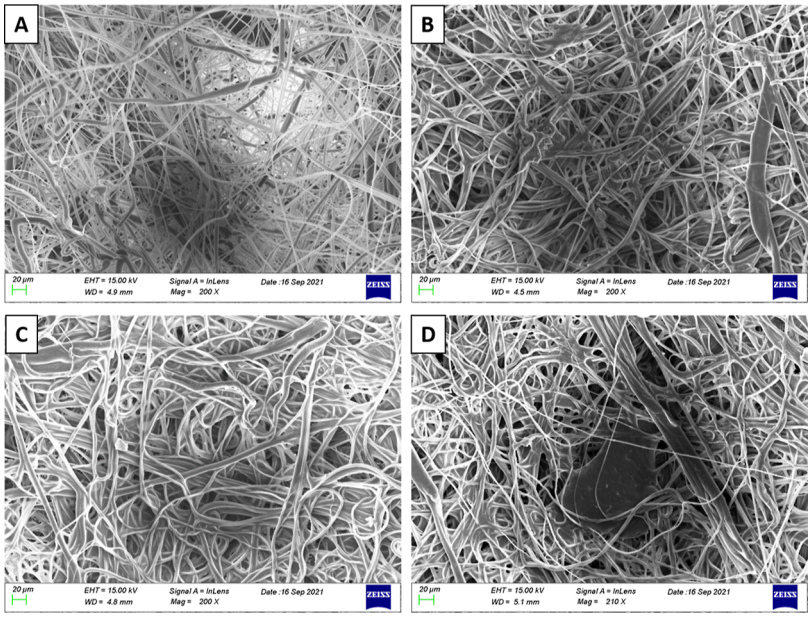

Figure 5. SEM images of the coated HEPA filters with P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w) of different loadings. (A) Control, (B) 8, (C) 20, and (D) 25%.

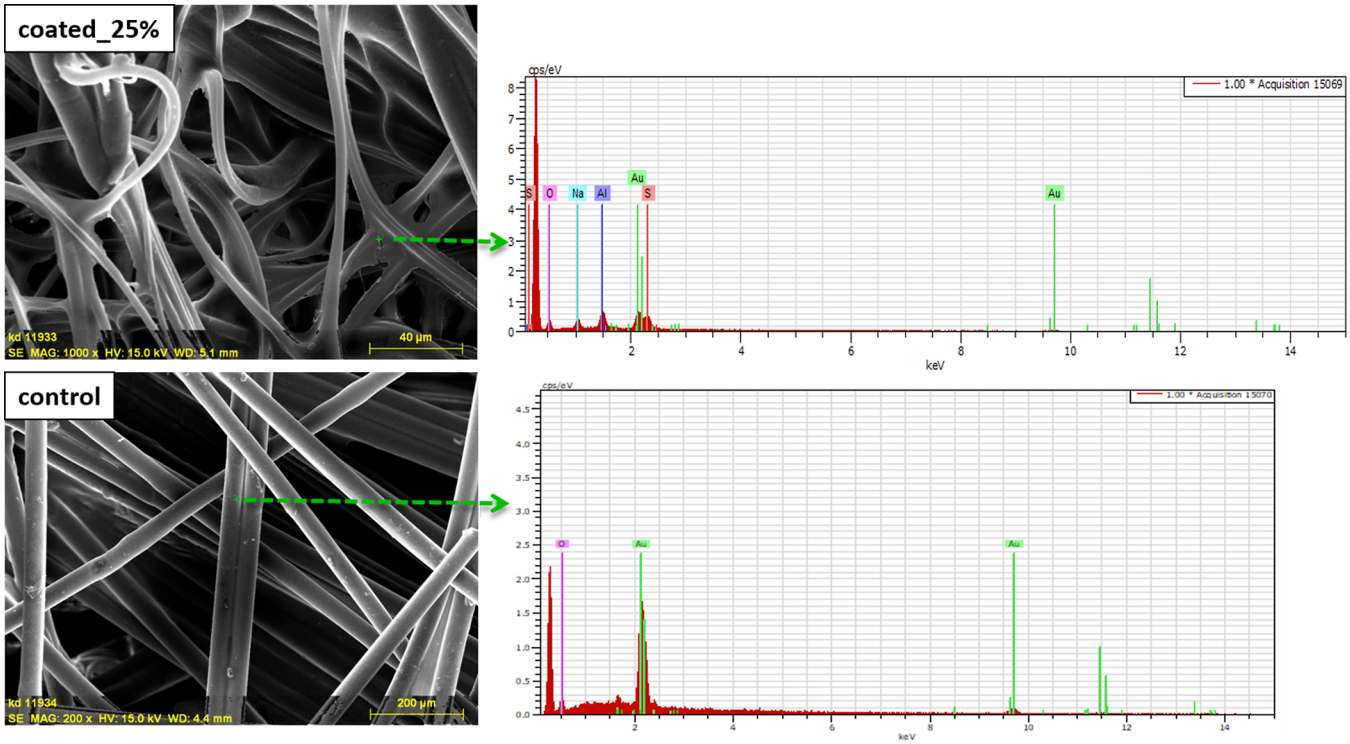

Figure 6. EDS analysis of the coated filter with P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w) of 25% loading (up) and of the uncoated-control filter (down) for comparison.

In order to get a better understanding of the reaction, a stoichiometric mixture of the initial copolymer P(SSNa-*co*-GMA20) with the homopolymer PDMAEMA (eq_{GMA}/$eq_{PDMAEMA}$ = 1) was prepared in aqueous solution (6% w/v) at room temperature and was characterized by ATR−FTIR spectroscopy. The corresponding w/w composition of the two polymers' mixture was 85/15%. As shown in the photograph of Figure 3, gelation of the solution occurred almost immediately after mixing, indicating the possible reaction between the polymers. An evidence of the cross-linking reaction is obtained from the ATR−FTIR study, where the peak at 909 cm^{-1}, corresponding to the epoxy ring of GMA, disappeared completely from the spectrum of the mixture, suggesting the practically quantitative opening of oxirane ring by the nucleophilic attack of the tertiary amine. Moreover, the bands at 2819 and 2766 cm^{-1} disappeared, indicating the possible quaternization of the tertiary amine of PDMAEMA.

Having verified the cross-linking reaction between the epoxide and the amine groups of the polymers, the next step was to study the behavior of the mixture after the introduction

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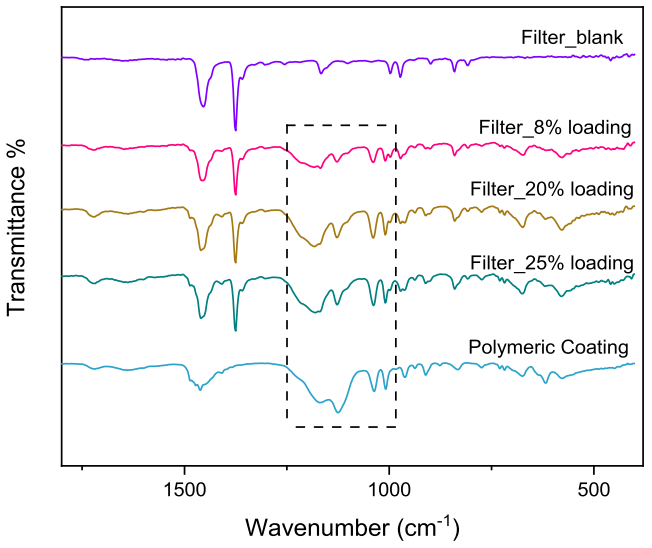

Figure 7. ATR−FTIR spectra of the filters coated with P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w) at different % loadings. The spectra of the uncoated filter and the polymeric material are shown for comparison.

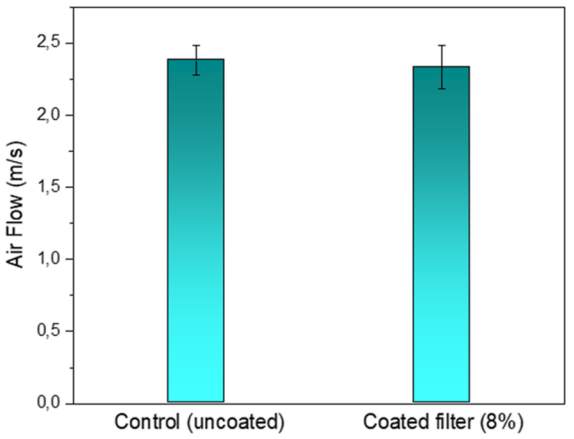

Figure 9. Average rate of air flow through the control (uncoated) HEPA filter and the coated filter (8% loading). The error bars represent the standard deviation of the means.

of the cetyl ammonium biocidal group. Therefore, an aqueous solution of the P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20) terpolymer was mixed with an aqueous solution of PDMAEMA in a composition 90/10% w/w ($eq_{GMA}/eq_{PDMAEMA}$ = 1) and the behavior of the aqueous mixture was followed over time for two different temperatures (4 and 25 °C) (Figure 4). At 25 °C, the viscosity of the initial marginally viscous aqueous mixture (∼6 mPa s) started to increase after 1 day, tending to reach a plateau value (2−2.5 Pa s), after 2 days. In fact, the frequency dependence of the loss (G'') and storage (G') moduli after gelation suggests that a chemically cross-linked hydrogel has been formed since G' is much higher than G'' and the two moduli are parallel without evidence of any cross-sectional tendency within the frequency range studied.

From the practical point of view, the gradual cross-linking at room temperature will complicate the final application since the aqueous polymer mixture should be prepared just prior to spray coating of HEPA filters. However, cross-linking is practically suppressed, at least for a few days, if the mixture is stored at 4 °C, as suggested by the viscosity results shown in Figure 4. Although these results should be further optimized, it seems that storing at low temperature offers an attractive alternative for potential industrialization of the coating process, when the in situ preparation of the aqueous mixture is not applicable.

3.4. Preparation and Characterization of Biocidal HEPA Filters. Biocidal functionalization of HEPA filters was achieved by spray coating with the polymeric aqueous solutions P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w). The treated filters and untreated control filters were imaged using SEM to evaluate the visible and structural differences (Figure 5). The images showed no significant differences between the control and the lowest polymeric loading of 8%, whereas in the case of the highest loading (25%), polymeric films appeared to cover some hollow areas of the filters. In addition, homogeneous modification of

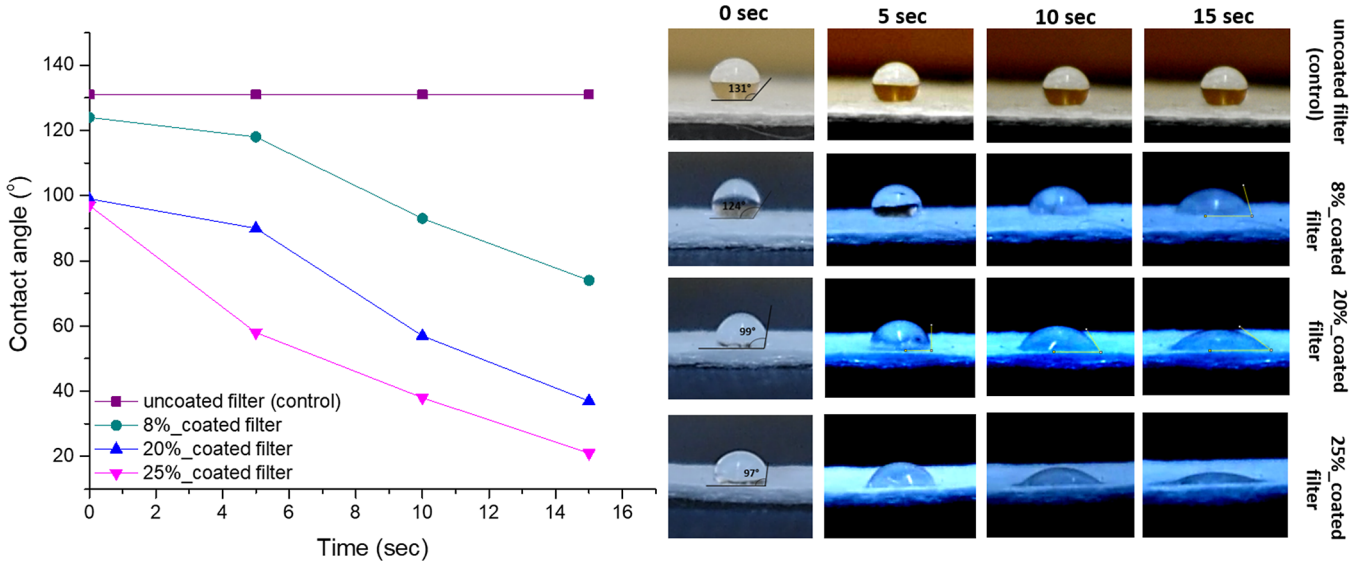

Figure 8. Evolution of water contact angles (WCAs) vs time for the filters coated with P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w) at different % loadings, as well as for the uncoated (control) filter.

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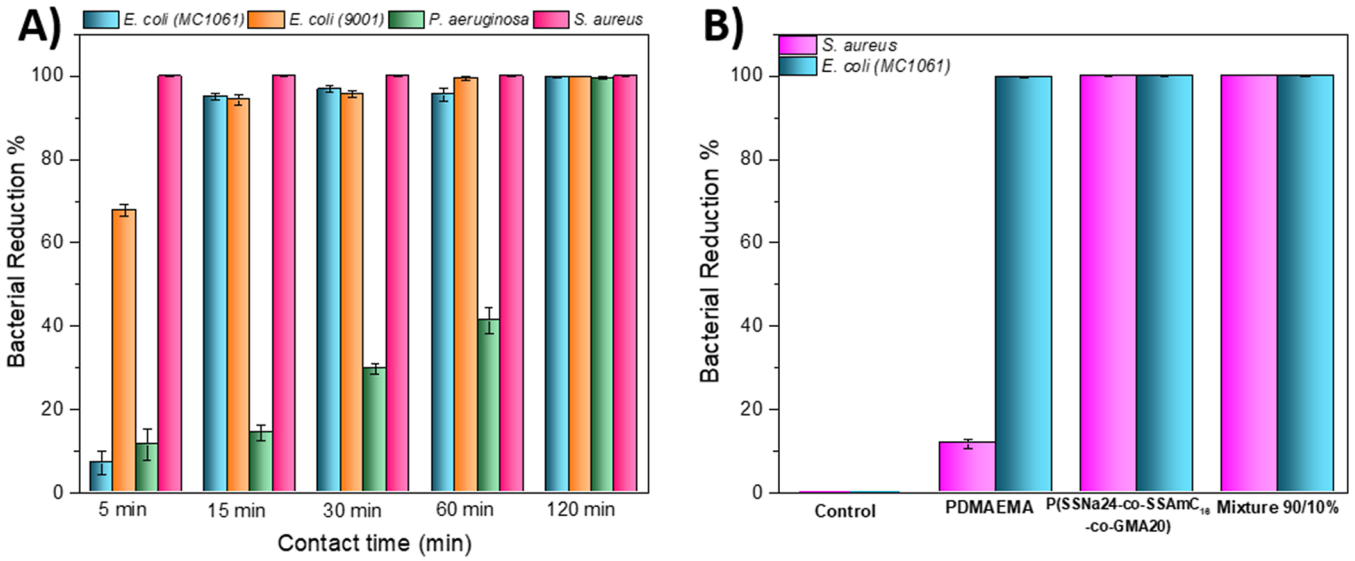

Figure 10. (A) Time-kill assay of the coatings against *E. coli* (Strains MC1061 and 9001), *P. aeruginosa*, and *S. aureus* showing their reduction % after different contact-time intervals (5, 15, 30, 60, and 120 min). (B) Bacterial reduction % of *E. coli* (MC1061) and *S. aureus* after contact with the initial polymers P(SSNa24-*co*-SSAmC16-*co*-GMA20)], PDMAEMA, and their 90/10% mixture, after 120 min contact.

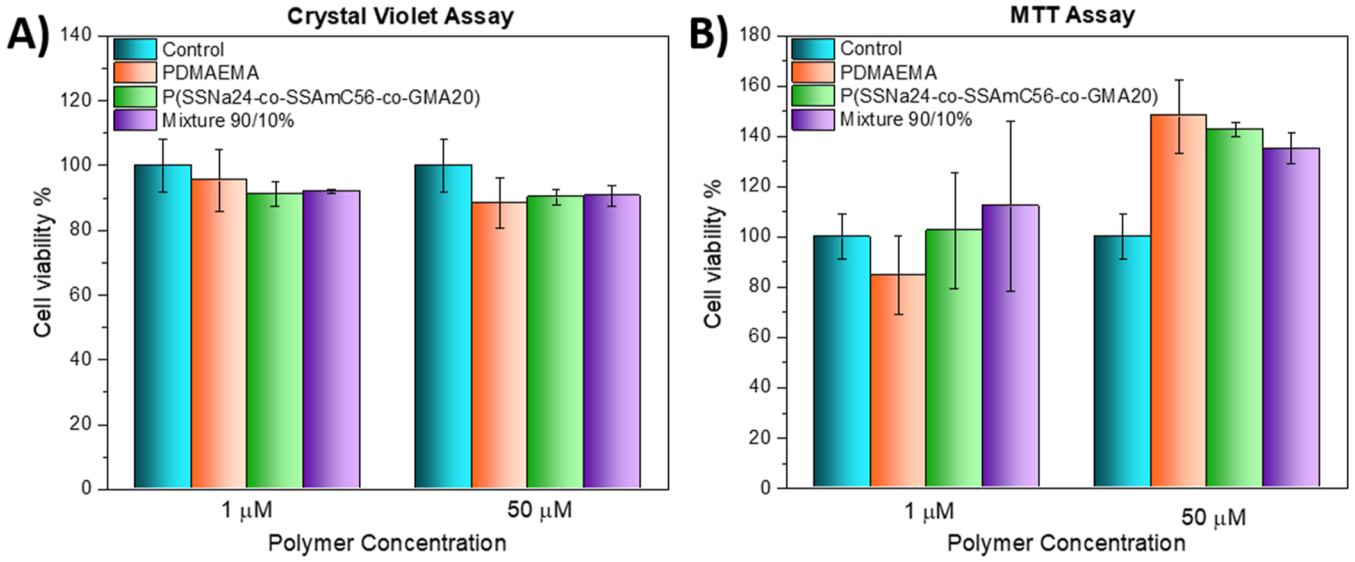

Figure 11. Effects of PDMAEMA, P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), and the mixture 90/10% (w/w) of them on cell viability. (A) Cell viability following pre-coating of cell culture plates with 1 and 50 μg/mL of each polymer and culture of A549 cells for 24 h. (B) Cell viability following 24 h treatment of A549 cells with 1 and 50 μg/mL of each polymer.

the fibers is observed in all loading levels, while the average fibers' diameter was not affected after the treatment at 8% loading, indicating that the efficiency of air flow may not be affected by the incorporation of the polymers to the fibers in this case.

To confirm that the surface of the filters was modified successfully by the polymeric material, EDX analysis was performed on the control (uncoated) and the coated filter with 25% loading (Figure 6). The presence of the polymeric material on the treated filter was confirmed mainly by the peaks responsible for S and Na elements of the terpolymer P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), whereas in the case of the uncoated filter, no such peaks were observed.

ATR−FTIR analysis was also performed on the control filter and the treated filter as well. In the spectra of Figure 7, it is clearly observed that there is a difference between the coated filters with the uncoated one. More specifically, as the loading increases, the peaks that are attributed to the polymeric material (1120−1180 and 1010−1040 cm^{-1}) appear more obviously in the spectra of the coated filters.

The surface of treated filters was further characterized by water contact angle measurements. As demonstrated in Figure 8, the filter's surface became less hydrophobic after functionalization with the biocidal polymeric materials in different % loadings. More specifically, as the % loading on the filter increased (from 0 to 25%), the contact angle of the water droplet decreased (131−97°), again confirming that the hydrophilic polymeric material P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20)/PDMAEMA 90/10% (w/w) is present on the HEPA surface. In the graph of Figure 8, the evolution of contact angles is displayed showing that the water droplet is quickly adsorbed on the coated filters, especially in the case of

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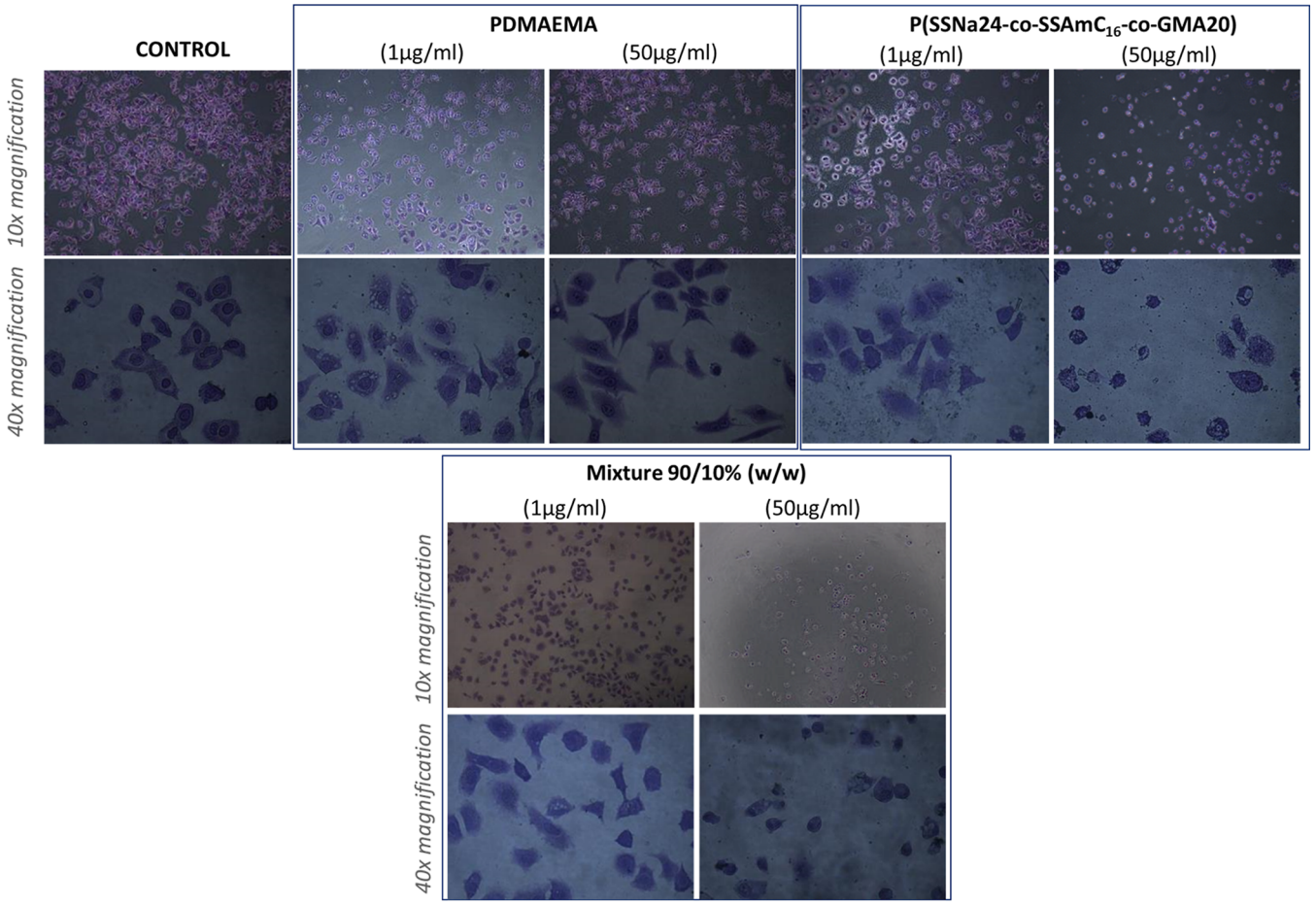

Figure 12. Phase contrast photographs with crystal violet dye of A549 cells cultured on pre-coated cell culture plates with 1 and 50 μg/mL PDMAEMA, P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), and the mixture 90/10% (w/w).

the highest loading (25%), where increased hydrophilicity was observed, with the contact angle reaching the lowest value of 21° after 15 s. This behavior is quite encouraging according to a very recent study,[55] where nanofibrous materials of Zn-modified PVDF were prepared for use as air filters. Interestingly, the results of that study indicated that the material with the highest hydrophilicity (high water adsorption) exhibited the highest virucidal activity.

The effect of polymeric coating on air permeability through the filter was examined to prove no alteration in the efficiency of air flow in the studied system. An uncoated (control) HEPA filter and a coated filter with the lowest loading (8%) were installed in an industrial unit, and the air flow across both filters was measured (Figure 9). The air flow across the control filter was 2.38 ± 0.1 m/s and across the coated filter it was 2.33 ± 0.15 m/s, indicating that there was no significant difference between them. Therefore, biocidal treatment of the HEPA filter with the polymeric materials through spray coating did not practically impede air permeability.

3.5. Antibacterial Activity of the Polymers. The evaluation of antibacterial efficiency of the polymeric mixture P(SSNa24-*co*-SSAmC$_{16}$56-*co*-GMA20)/PDMAEMA 90/10% for contact times of 5, 15, 30, 60, and 120 min is presented in Figure 10A. In the case of *P. aeruginosa*, antibacterial efficacy required a more protracted contact (2 h). All other species were killed faster, already from the first 15 min. The most sensitive was *S. aureus* which was inhibited completely after 5 min of contact with the polymer. The selective toxicity of a polymer bearing releasable ammonium cationic groups against Gram-positive bacteria has been observed before.[56] The cytoplasmic membranes of Gram-positive bacteria are surrounded by a rigid peptidoglycan layer with plenty of pores that may allow external small molecules (such as CTAB) to come in contact with the cytoplasmic membrane leading to cell lysis.

A contact time of 120 min was chosen to determine the antibacterial nature of the separate polymers P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), PDMAEMA, and their 90/10% w/w mixture. The cell viability of *E. coli* and *S. aureus* is displayed in Figure 10B. The PDMAEMA homopolymer inhibited the growth of *E. coli* but had no effect against *S. aureus*. As suggested by the literature,[57] the less hydrophilic nature of PDMAEMA allows for more efficient interaction with the outer membrane of the Gram-negative bacteria leading to their permeabilization. On the other hand, the terpolymer P-(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20) inhibited both bacterial species efficiently.

3.6. Toxicological Assessment of the Polymeric Materials. The potential cytotoxic effects of the herein developed polymeric coatings were also evaluated following in vitro exposure since cytotoxicity tests are considered necessary to define the potential risks and fates of the exposure to newly synthesized compounds.[58] The cytotoxicity tests were performed in the A549 lung adenocarcinoma in vitro model by following two approaches to better address the effects of polymer exposure. In the first approach, cell culture plates were

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first coated with 1 and 50 μg/mL of PDMAEMA, P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), and the mixture 90/10% (w/w) overnight and cells were added on the top of the coating in a 2% culture medium, cultured for 24 h, and cell viability was assessed. In the second approach, each polymer had been dissolved in H_2O, cells were treated for 24 h in serum-free conditions in the presence or absence of the polymers, cell viability assay had been evaluated and cell morphology had also been monitored.

We first studied the effects on cell viability of PDMAEMA, P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), and the mixture 90/10% (w/w) of them. The concentration range of each polymer was 1−50 with 10 μg/mL increments (the low and high concentrations are presented). As shown in Figure 11A, no statistically significant changes in cell viability were observed following pre-coating of cell culture plates with the polymers and culture of A549 cells for 24 h, as compared to the untreated (control) cells. As far as the second approach for studying cell viability is concerned, treatment of A549 cells with each polymer for 24 h resulted in no statistically significant cytotoxicity in terms of cell viability as compared to the control A549 cells (Figure 11B). Intriguingly, it seems that this cell culture model favors the highest concentration (50 μg/mL) of the tested materials.

Regarding the cell morphology, as shown in Figure 12, no changes were found in cell morphology following coating with PDMAEMA, while only in high concentration of P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20) and the mixture 90/10% (w/w), slight changes in cell morphology were observed.

We can, therefore, conclude that the in vitro study of cytotoxicity reveals no significant health risk of PDMAEMA, P(SSNa24-*co*-SSAmC$_{16}$-*co*-GMA20), and the mixture 90/10% (w/w) used for the development of biocidal air filters in the concentration range tested.

4. CONCLUSIONS

In this study, water-soluble biocidal copolymers bearing quaternized ammonium groups were synthesized and further used as coatings on HEPA filters to prevent the high microbial contamination that could act as a secondary source of air pollution. Aiming at a more environmentally friendly approach, the whole process was conducted using only water as the solvent. Thus, initial copolymers were synthesized by water-based polymerizations, while active coatings were also obtained in the form of aqueous solutions. The filters were treated with the polymeric solutions through spray coating, and the properties of the modified filter's surface were examined. An important point is that no impedance was shown on the air flow through the coated filter. The developed coatings exhibited rapid killing efficiency against most of the examined Gram-positive and Gram-negative bacterial species (only 5 min for *S. aureus* and 15 min for *E. coli*), while the cytotoxicity study of the polymeric materials on A549 cells revealed no health risk. Overall, a facile and eco-friendly methodology was developed for the construction of HEPA with biocidal properties, after treatment with antimicrobial water-based polymeric materials by the spray-coating method. Such biocidal-treated filters can be great candidates for practical use in air-purification applications for prevention of bacterial or viral infections.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c04427.

Growth curves of the studied bacterial strains and ATR−FTIR and ^{1}H NMR characterization of the synthesized polymers (PDF)

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Author Contributions

D.D. and I.T. contributed equally to the experimental part of the synthesis and analysis of polymeric materials. Z.P. and K.T. performed the experimental part of cytotoxicity tests. D.T. conceived the main idea, and J.K.K. supervised the research and reviewed the manuscript. D.D. designed and wrote the main manuscript text, and G.B., A.V.-G., N.K., and Z.P. contributed to specific sections. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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