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# Antimicrobial Activities of Hydrophobically Modified Poly(Acrylate) Films and Their Complexes with Different Chain Length Cationic Surfactants

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**Abstract:** Multilayer films from hydrophobically modified poly(acrylic acid) (HMPA) and their complexes with cationic surfactants were successfully prepared using the layer-by-layer (LbL) method. Alkyl trimethylammonium bromide derivatives with various lengths of the hydrophobic chain ($C_{10}$–$C_{18}$) were used to interact with the HMPA polymer, generating highly hydrophobic domains in the films and contributing to the antimicrobial properties of the prepared coating. The antimicrobial efficiency against common pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* was investigated in relationship with the morphology and composition of the thin films. The wettability and roughness of the multilayered systems were evaluated using atomic force microscopy (AFM) and contact angle measurements. The effects of the microbial exposure on the surface properties of the prepared films were investigated in order to assess the stability of the HMPA-deposited multilayers and the durability of the antimicrobial activity. The hydrophobically modified films exhibited antimicrobial activity against the studied pathogens. The best efficiency was registered in the case of *S. aureus*, which showed an inhibition of growth up to 100% after 2 h. *C. albicans* proved to be less sensitive to the effect of the multilayers deposited from HMPA–surfactant complexes. These results suggest that HMPA and HMPA–surfactant complex LbL multilayer films can be used as promising materials in antimicrobial surface coatings with increased resistance to pathogens during exposure.

**Keywords:** antimicrobial activity; hydrophobically modified poly(acrylate); layer-by-layer deposition; water contact angle; film roughness

## 1. Introduction

Polymeric materials are widely used as antimicrobial nanocoatings to prevent biocontamination, which has a direct impact on human health. In recent years, polymeric thin films, membranes,

and scaffolds have proved their usefulness in medical device fabrication, drug delivery, and health care products, along with other major applications in textiles, food packaging, water decontamination, and information technologies applied to diagnostic methods [1–7]. Many strategies and materials have been developed to obtain efficient self-cleaning and/or antimicrobial coatings [8]. The antimicrobial efficiency of a thin film is mainly related to the chemical composition, while the self-cleaning effect is due to its morphology. Thus, both the material and the preparation method influence the biological behavior of polymeric nanostructured films.

The layer-by-layer (LbL) self-assembly technique is a useful method to prepare highly hydrophobic and/or antimicrobial multilayer films due to the simplicity of the experimental conditions. It also allows extended variation of properties by evaluating molecular interactions between the different types of materials used and tuning the composition [9,10]. The low cost of preparation and the opportunity to control the composition and morphology, leading to improved structural, electrical, optical, and antimicrobial properties, make the self-assembly method the most attractive approach for the fabrication of films in biomedical applications [1–11]. When a multilayer film is obtained, the existing molecular interaction will generate new properties which allow applications on different materials. Moreover, it is possible to combine different properties of certain materials to create new synergetic systems, such as superhydrophobic architectures with antibacterial or antifungal properties.

Among various materials of interest used for the fabrication of self-assembled films, poly(acrylate) (PANa) is an intrinsically conducting polymer which possesses electrical, optical, and anticorrosive properties [12]. In addition, hydrophobically modified poly(acrylate) (HMPA) films exhibit high chemical and environmental stability; ease of manipulation, polymerization, and doping; and low-cost production [13]. Gratzl et al. found that the presence of acid-form of polyacrylic acid (PAA) in the polymer and slightly acidic conditions are important for good antimicrobial activities of the material, while counterions reduce its potency dramatically [14].

Cationic surfactants, consisting of a hydrophilic quaternary ammonium group and a hydrophobic alkyl chain, have strong bactericidal potential and are frequently used for disinfection in a variety of fields, such as medicine and food manufacturing. Moreover, cationic surfactants such as alkyltrimethylammonium bromide possess the same activity for both Gram-positive (*Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-negative (*Staphylococcus aureus*) bacteria, according to Campanha et al. [15]. Surfactants with quaternary ammonium groups were considered relatively safe antimicrobial agents, but their increased usage in the last decades as disinfection and sanitation agents led to the emergence of the pathogens resistant to cationic surfactants.

Extensive studies have been performed to address the challenges associated with the application of cationic compounds against resistant bacterial infections. Recent papers report the synthesis of other antimicrobial compounds bearing ammonium groups, such as gemini cationic surfactants [16] or modified polymers [17,18].

Our approach in the present research was based on the advantage of using the very good antimicrobial properties of quaternary ammonium surfactants while increasing their stability and efficiency by incorporating them into modified hydrophobic polymer films.

Among other polymeric compounds used in the fabrication of LbL films, poly(diallyldimethylammonium chloride) (PDADMAC) exhibits only a weak antimicrobial effect due to the quaternary nitrogen which is pendant away from the backbone chain. It is reported that PDADMAC requires the presence of hydrophobic groups and a high charge density for efficient biocidal effect [19].

In our previous work, we obtained LbL films by electrostatic interaction between hydrophobically modified sodium poly(acrylates) ($PAC_nNa$) with and without cationic surfactants and PDADMAC [20,21]. Our present study is focused on the antimicrobial activity of hydrophobically modified HMPA and HMPA–surfactant complex LbL multilayer films. To the best of our knowledge, this is the first work to investigate the antimicrobial activity of HMPA–surfactant architectures obtained using the LbL technique. The antimicrobial activity of films based on $PAC_{18}Na$-$C_xTAB$ ($x$ = 10, 12, 14, and 18) were tested against *S. aureus*, *E. coli*, *P. aeruginosa* and *Candida albicans* as model microbial strains.

## 2. Materials and Methods

### *2.1. Materials*

Decyl-($C_{10}$TAB), dodecyl-($C_{12}$TAB), tetradecyl-($C_{14}$TAB), and octadecyltrimethylammonium bromide ($C_{18}$TAB) from Fluka (Waltham, MA, USA). Chemicals products were used as purchased (98%, 98%, 99%, and 97% purity).

Poly(ethylenimine) (PEI) aqueous solution (50 wt.%) with $M_W$ = 75,000, and poly(diallyldimethylammonium chloride) (PDADMAC) aqueous solution (23 wt.%) with $M_W$ 100,000–200,000 were supplied by Sigma-Aldrich (St. Louis, MO, USA).

Hydrophobically modified sodium polyacrylates, $PAC_n$Na ($n$ = 18), were obtained in our laboratory, according to the procedure reported in a previous study [22].

### *2.2. Preparation of Hydrophobically Modified Poly(Acrylate)–Surfactant Films*

The hydrophobically modified polymer $PAC_{18}$Na used for the fabrication of multilayer films was prepared according to the synthesis previously reported, together with the characterization of the prepared polymers [22]. The polymer–surfactant complexes $PAC_{18}$Na-$C_x$TAB ($x$ = 10, 12, 14 and 18) were prepared according to our previous study [20], and the aqueous $PAC_{18}$Na solution was added to a $C_x$TAB micellar solution under continuous stirring. The complexes were formed due to the electrostatic interaction between the negatively charged $PAC_{18}$Na and the positively charged $C_x$TAB. To ensure that the complexes were formed, measurements of zeta potential were carried out as in our previous paper [18]. The polymer–surfactant complexes ($PAC_{18}$Na-$C_n$TAB), ($10^{-2}$ M solution containing 0.01 M NaCl), PDADMAC ($10^{-2}$ M), and PEI ($5 \times 10^{-2}$ M) were prepared and stored in a refrigerator for further use. The multilayers were deposited alternatively on a glass previously treated with PEI by using PDADMAC and $PAC_{18}$Na solutions.

The LbL depositions were achieved using a programmable dipping machine (Dipping Robot DR-3, Riegler & Kirstein GmbH, Potsdam, Germany). The dipping procedure was reported elsewhere [20,21]. After six months of aging at room temperature and in the dark, the investigated films were exposed to a microbial environment. The composition of polyelectrolyte–surfactant films exposed to a microbial environment is presented in Table 1.

**Table 1.** Hydrophobically modified poly(acrylate)–surfactant films investigated for antimicrobial properties.

| No. | Sample | Surfactant Concentration (M) |
|---|---|---|
| 1 | $PAC_{18}$Na/PDADMAC | 0 |
| 2 | $PAC_{18}$Na-$C_{10}$TAB-NaCl/PDADMAC | $2 \times 10^{-2}$ |
| 3 | $PAC_{18}$Na-$C_{12}$TAB-NaCl/PDADMAC | $7 \times 10^{-3}$ |
| 4 | $PAC_{18}$Na-$C_{14}$TAB-NaCl/PDADMAC | $2 \times 10^{-3}$ |
| 5 | $PAC_{18}$Na-$C_{16}$TAB-NaCl/PDADMAC | $1 \times 10^{-3}$ |

The multilayer films consisted of 20 bilayers of alternatively deposited polyelectrolyte–surfactant complexes and PDADMAC. In our previous study, we observed that a sudden increase of contact angle (CA) occurred up to five bilayers. In order to save time and materials, we decided to use films containing 20 bilayers. These films have a good contact angle and also a rough surface, as the atomic force microscopy (AFM) results suggest after six months of aging [21].

### *2.3. Antimicrobial Activity Testing*

The antimicrobial activity of the polyelectrolyte–surfactant LbL films was tested against *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 10231 according to the National Committee for Clinical Laboratory Standards [23]. All the experiments were performed in triplicate. Fresh microbial cultures obtained on nutrient agar were used to make 0.5 McFarland suspensions that were further diluted till a working solution of $1.5 \times 10^6$ colony forming units (CFUs).

The films (initially sterilized by exposure for 30 min to UV on either side) were incubated in tubes with 9 mL of saline water and 1 mL of bacterial suspension at 37 °C under continuous shaking. After different periods of time (right after initial contact, 2, 3, 4, 5, 6 and 24 h, respectively), the number of CFUs was determined.

### 2.4. Characterization Methods

The contact angle measurements were collected using a Drop Shape Analysis System, model DSA1 (FM40 Easy Drop) from KRÜSS GmbH (Hamburg, Germany). The water drop volume was of 3 mL, and the measurements were done in static regime at room temperature in air. Zeta potential measurements were carried out on a MalvernNano ZS Zetasizer (Malvern Ltd., Malvern, UK) at room temperature to measure the electrokinetic potential. AFM measurements were carried out in noncontact mode with an XE-100 (Park Systems, Suwon, Korea) equipped with flexure-guided, cross-talked eliminated scanners, as recommended for soft materials. The morphological AFM images were taken at $2 \times 2$ $\mu m^2$. The XEI program (v.1.8.0) was used for image processing and roughness evaluation. In order to have a better view of the surface morphology, the AFM images are presented in the so-called "enhanced color view mode".

## 3. Results and Discussion

From the LbL deposition method, thin films consisting of a mixture of hydrophobically modified polymers and surfactants were obtained. Various surfactants with hydrocarbon chains from $C_{10}$ to $C_{18}$ were used in order to improve the antimicrobial activity and to modify the morphology of the thin film. We observed that the contact angle increased suddenly after the deposition of five bilayers. Moreover, after aging six months, the contact angle and the rough surface increased significantly for the films containing 20 bilayers according to our study [20].

Also, our previous study evidenced that a PDADMAC outer layer leads to ball-like particles being randomly deposed [20]. Consequently, the obtained films presented higher roughness and contact angle compared with the films without a PDADMAC outer layer. In order to observe if one or more layers were removed from the polyelectrolyte multilayer films, zeta potential measurements were conducted. The zeta potential data proved to be negative for all of the samples. The results are presented in Table 2.

**Table 2.** Zeta potential data for several deposed films.

| Time (h) | Zeta Potential (mV) | | | | |
|---|---|---|---|---|---|
| | $PAC_{18}$Na-NaCl/PDADMAC | $PAC_{18}$Na-$C_{10}$TAB-NaCl/PDADMAC | $PAC_{18}$Na-$C_{12}$TAB-NaCl/PDADMAC | $PAC_{18}$Na-$C_{14}$TAB-NaCl/PDADMAC | $PAC_{18}$Na-$C_{18}$TAB-NaCl/PDADMAC |
| 0 | −48.1 | −10.4 | −19.39 | −15.63 | −15.51 |
| 2 | −55.8 | −17.27 | −19.9 | −18.8 | −20.2 |
| 4 | −41.4 | −16.6 | −26.6 | −16.7 | −26.4 |
| 6 | −41.4 | −18.5 | −29.8 | −19.4 | −25.3 |
| 24 | −39.8 | −17.8 | −27.6 | −18.42 | −25.1 |

The stability of the thin films in aqueous solution was investigated and the results are shown in Table 2. The samples were kept for 24 h at room temperature to observe if the electrokinetic potential changed during the experiment. After 2 h of stabilization, zeta potentials reached values that did not change significantly (~1 to 2 mV) during the rest of the 22 h of storage in aqueous solution. Results indicated that the surfaces did not seem to lose polymer even when the polyelectrolyte or polyelectrolyte–surfactant films were immersed for 1 day in liquid media. The results were similar with the behavior previously observed by Costerton et al. for a multilayered architecture immersed in water, MeOH, hexanes, acetone, dichloromethane, and ethyl acetate [24].

### 3.1. Evaluation of the Antimicrobial Activity of Multilayered Films

In order to evaluate the antimicrobial activity of multilayered films, *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* were selected as model microorganisms, as they are responsible for the most frequent hospital infections. The antimicrobial properties were investigated by testing the bacterial growth in liquid broth media. Figure 1 presents the bacterial growth of *E. coli* in liquid media on polymer–surfactant complex films of $PAC_{18}Na$-$C_x$TAB-NaCl/PDADMAC ($x$ = 10, 12, 14, and 18) compared to the results of bacterial growth on $PAC_{18}Na$-NaCl/PDADMAC and untreated glass. On the control material (untreated glass), the bacteria showed a constant growth rate after 24 h of exposure. The antimicrobial activity of all the tested materials (polyelectrolyte–surfactant films and pristine $PAC_{18}Na$ film) on *E. coli* proved to be very good after 24 h of incubation, as demonstrated by the complete bacterial growth inhibition. An unexpectedly high efficiency was recorded for the film prepared with the $C_{14}$TAB surfactant $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, since no CFUs after 3 h of contact were present on the tested multilayers.

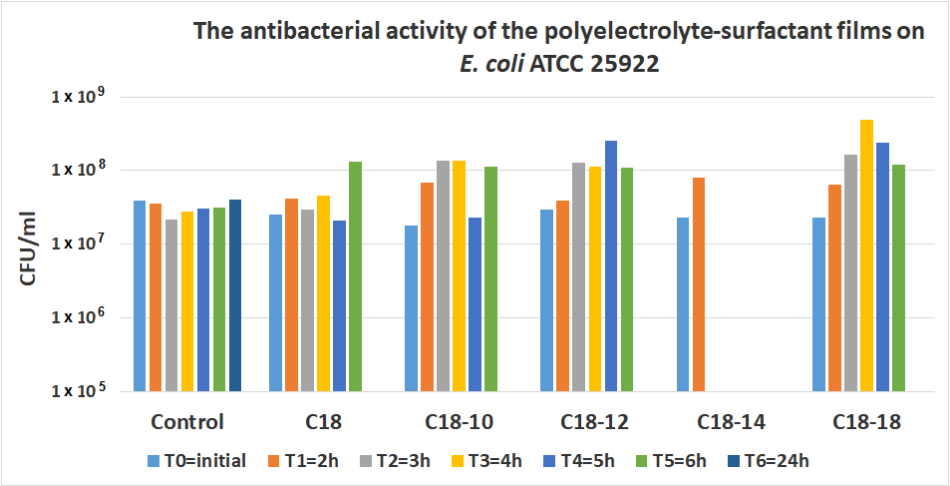


**Figure 1.** The antibacterial activity of the polyelectrolyte–surfactant films on *Escherichia coli* ATCC 25922 after different times of contact (initial, 2, 3, 4, 5, 6, and 24 h): (control—clear glass, C18-$(PAC_{18}Na/PDADMAC)_{20}$, C18-10-$(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$, C18-12-$(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$, C18-14-$(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, and C18-18-$(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$).

Even though *Pseudomonas aeruginosa* is also a Gram-negative bacteria just like *E. coli*, it was more resistant to the antimicrobial activity of the films. $(PAC_{18}Na\text{-}NaCl/PDADMAC)_{20}$ and $(PAC_{18}Na\text{-}C_{10}TAB\text{-}NaCl/PDAMAC)_{20}$ inhibited the strain's development after 24 h and $(PAC_{18}Na\text{-}C_{18}TAB\text{-}NaCl/PDAMAC)_{20}$ film presence determined a colony forming unit (CFU) decrease after 5 and 6 h of incubation compared with the control sample (Figure 2).

The multilayers prepared from mixtures of hydrophobically modified polymers and cation surfactants with longer alkyl chains ($C_{12}$–$C_{18}$) exhibited no antibacterial activity. The films from polymer $(PAC_{18}Na/PDADMAC)_{20}$ without a surfactant and with a short chain surfactant ($C_{10}$) exhibited the inhibition of bacterial growth after 24 h of exposure.

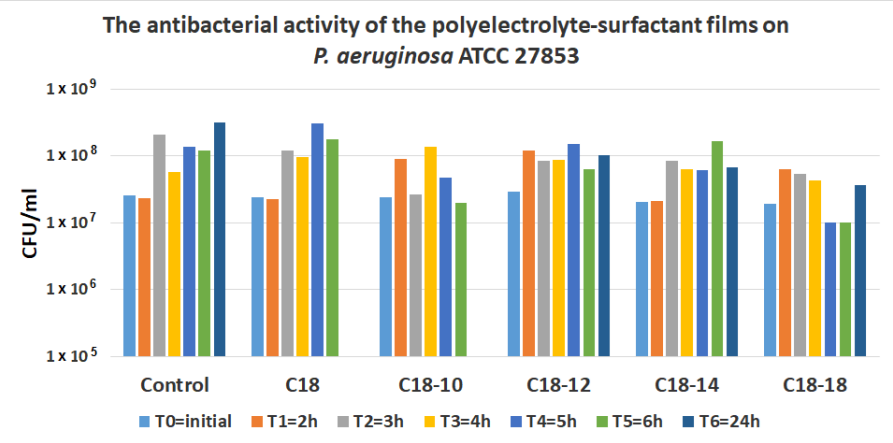


**Figure 2.** The antibacterial activity of the polyelectrolyte–surfactant films on *P. aeruginosa* ATCC 27853 at different times of contact (initial, 2, 3, 4, 5, 6, and 24 h): (control—clear glass, C18-$(PAC_{18}Na/PDADMAC)_{20}$, C18-10-$(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$, C18-12-$(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$, C18-14-$(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, and C18-18-$(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$).

In Figure 3, the development and survival of *S. aureus* strains in the presence of the films containing hydrophobically modified polyacrylate and the complexes with various alkyltrimethylammonium bromide at 20 bilayers are shown. All the multilayers exhibited a significant inhibition of bacterial growth after 2 or 3 h of incubation, while the film $(PAC_{18}Na/PDADMAC)_{20}$ without a surfactant proved to be the most effective.

The fungal strain *C. albicans* was less susceptible to the films' activity, as can be observed in Figure 4, and the number of CFUs was constant for all the tested samples. The exception was the film prepared with the $C_{14}$ surfactant, which showed moderate activity: 24 h of exposure resulted in the inhibition of fungal growth.

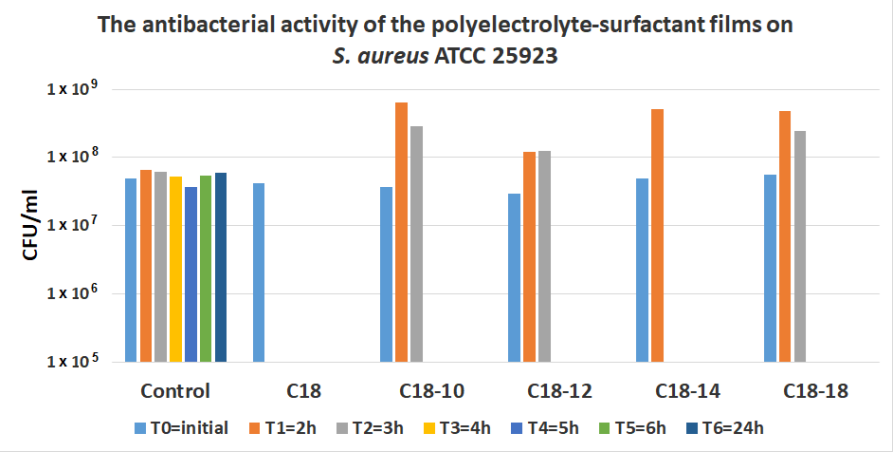


**Figure 3.** The antibacterial activity of the polyelectrolyte–surfactant films on *Staphylococcus aureus* ATCC 25923 at different times of contact (initial, 2, 3, 4, 5, 6, and 24 h): (control—clear glass, C18-$(PAC_{18}Na/PDADMAC)_{20}$, C18-10-$(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$, C18-12-$(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$, C18-14-$(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, and C18-18-$(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$.

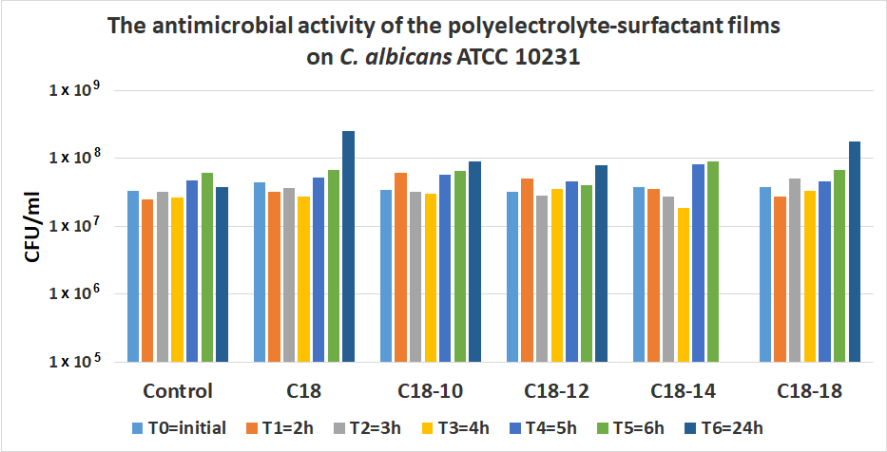


**Figure 4.** The antimicrobial activity of the polyelectrolyte–surfactant films on *Candida albicans* ATCC 10231 at different times of incubation (initial, 2, 3, 4, 5, 6, and 24 h): (control—clear glass, C18-$(PAC_{18}Na/PDADMAC)_{20}$, C18-10-$(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$, C18-12-$(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$, C18-14-$(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, and C18-18-$(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$).

### 3.2. Effect of Bacterial Growth on the Multilayered Hydrophobic Film Properties

In order to observe if the bacterial media affected the wettability of the films, contact angle measurements were performed. In Table 3, the contact angle values for untreated multilayer films and those exposed to bacterial media are presented.

**Table 3.** The contact angle (CA) values for untreated films and films subjected to microbial attack with positive and negative bacteria.

| Sample | CA for Untreated Films (°) | CA for Films Treated with *E. coli* (°) | CA for Films Treated with *S. aureus* (°) | CA for Films Treated with *P. aeruginosa* (°) |
|---|---|---|---|---|
| Clear glass | 26 | 25 | 23 | 24 |
| $(PAC_{18}Na/PDADMAC)_{20}$ | 93 | 90 | 97 | 85 |
| $(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$ | 101 | 98 | 108 | 90 |
| $(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$ | 103 | 103 | 115 | 96 |
| $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$ | 107 | 105 | 119 | 98 |
| $(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$ | 124 | 126 | 122 | 113 |

As expected, the results show that the increase of the alkyl chain length of the surfactant in the composition of the multilayer films led to higher contact angles. The CA values determined on the films incubated with *E. coli* and *S. aureus* did not show significant changes, while the presence of *P. aeruginosa* produced a decrease of the hydrophobicity of the films prepared with all surfactants.

Figure 5 presents the water contact angle profiles for hydrophobic layer-by-layer films based on modified poly(acrylate) and their complexes with cationic surfactants. As can be observed, the experimental data demonstrated that the contact angle increased with the alkyl chain length of the polymer graft and furthermore obeyed the trend previously observed for unaged or freshly prepared films [18,19]. In addition, the measured contact angle of the films subjected to a bacterial environment had almost the same values. For example, the contact angle of $(PAC_{18}Na\text{-}NaCl/PDADMAC)_{20}$ before bacterial exposure was 93°, while the contact angle for the polyacrylate films exposed to *E. coli* was 90°. For $(PAC_{18}Na\text{-}C_{12}TABNaCl/PDADMAC)_{20}$ exposed to bacteria, the contact angle remained the same, namely, 103°.

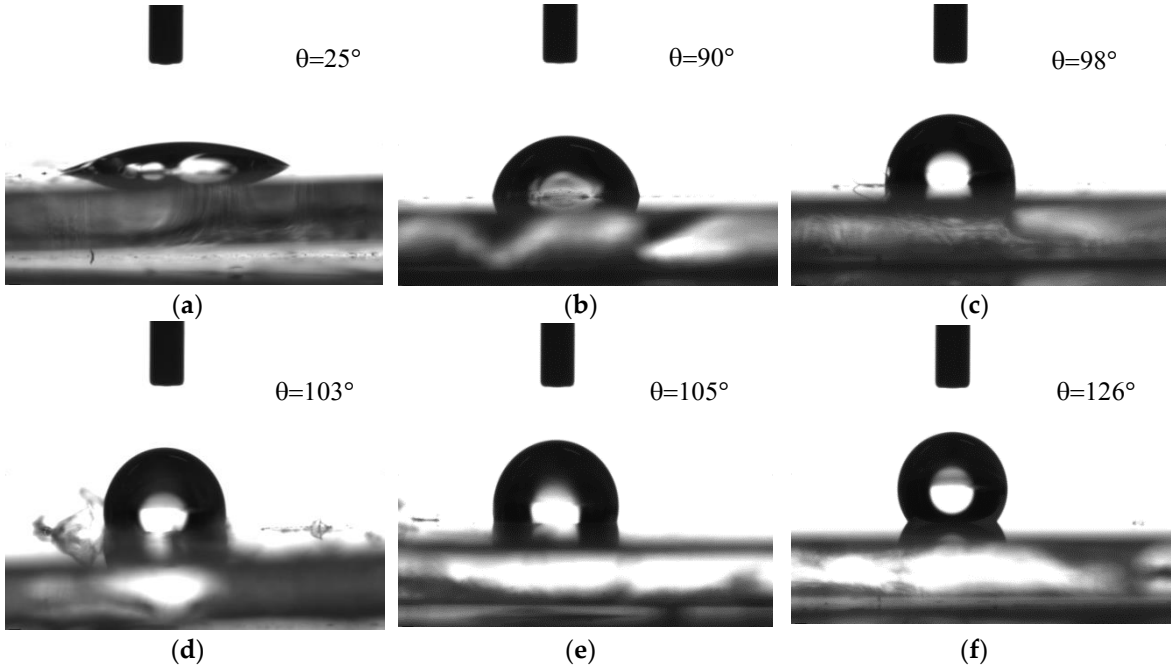


**Figure 5.** Water drop contact angle profile for: (**a**) clear glass, (**b**) $(PAC_{18}Na/PDADMAC)_{20}$, (**c**) $(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$, (**d**) $(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$, (**e**) $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$ and (**f**) $(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$ multilayer films exposed to *E. coli* ATCC 25922.

The wettability results of polyelectrolyte films exposed to *S. aureus* cells are presented in Figure 6. The contact angle of the untreated $(PAC_{18}Na/PDADMAC)_{20}$ was 93°, while for $(PAC_{18}Na/PDADMAC)_{20}$ exposed to *S. aureus*, the contact angle was calculated to be 97°. For $(PAC_{18}Na\text{-}C_{18}TAB/PDADMAC)_{20}$ exposed to *S. aureus*, the contact angle was 122°, and for the uninfected one, CA = 124°.

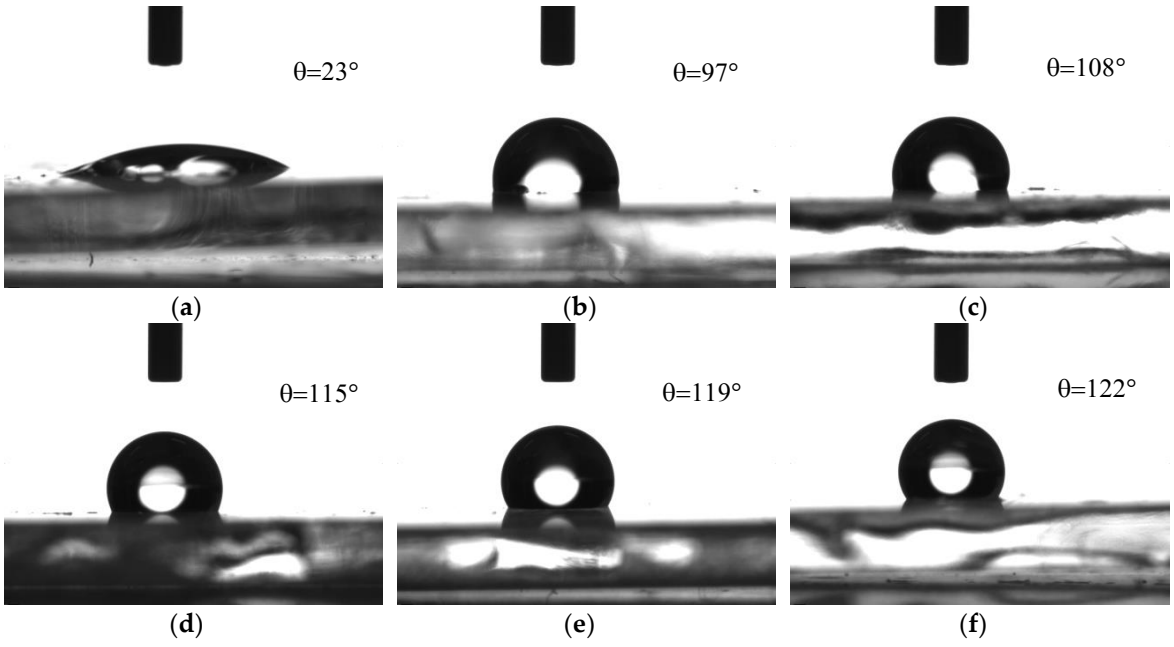


**Figure 6.** Water drop contact angle profile for: (**a**) clear glass, (**b**) $(PAC_{18}Na/PDADMAC)_{20}$, (**c**) $(PAC_{18}Na\text{-}C_{10}TAB/PDAMAC)_{20}$, (**d**) $(PAC_{18}Na\text{-}C_{12}TAB/PDAMAC)_{20}$, (**e**) $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$ and (**f**) $(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$ multilayer films exposed to *S. aureus* ATCC 25923.

### *3.3. The Morphology of the Films after Exposure to Bacterial Media*

AFM measurements were performed in order to demonstrate the possible film structure modifications occurring after exposure to bacterial media. Figure 7 presents the AFM images of multilayer films exposed to *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *P. aeruginosa* and without bacteria. The morphological measurements for the untreated multilayer films of $(PAC_{18}Na/PDADMAC)_{20}$ (Figure 7$a_0$), $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$ (Figure 7$b_0$), and $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$ (Figure 7$c_0$) reveal a rough morphology wherein air bubbles could be locked. The presence of the alkyl chain length indicated a higher root-mean-square (RMS) roughness and contact angle [19]. The AFM images of multilayer films exposed to *E. coli* ATCC 25922 indicated an irregular film structure, consisting of hills and valleys. The line scan was located within 0.9 nm for $(PAC_{18}Na/\ PDADMAC)_{20}$ (Figure 7$a_1$), 2 nm for $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$ (Figure 7$b_1$), and 6 nm for $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$ (Figure 7$c_1$). The RMS roughness for $(PAC_{18}Na/\ PDADMAC)_{20}$ was 0.290 nm, while for $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$, it was 0.394 nm. For $PAC_{18}Na\text{-}C_{18}TAB/PDADMAC$ at 20 bilayers, the RMS was 5.845 nm. When comparing the fresh multilayer film [19] with the ones incubated in a bacterial environment, we noticed that the bacterial growth did not change the surface morphology. These data confirmed our supposition that multilayer films are resistant to the bacterial attack of *E. coli*.

In order to observe the influence of *S. aureus* on multilayer films, AFM measurements were performed. Figure 7$a_2$,$b_2$,$c_2$ presents two-dimensional topographic AFM images at the scale of $2 \times 2\ \mu m^2$ of the glass substrate coated with $PAC_{18}Na/PDADMAC$, $PAC_{18}Na\text{-}C_{14}TAB/PDADMAC$, and $PAC_{18}Na\text{-}C_{18}TAB/PDADMAC$, respectively, together with characteristic surface profiles (line scans) along the horizontal direction. All films presented a uniform morphology consisting of hills and valleys, which, according to the line scans, were located within 1 nm (vertical direction) for the $PAC_{18}Na/PDADMAC$ film and 6 nm for the $PAC_{18}Na\text{-}C_{18}TAB/PDADMAC$ film, respectively. Another important feature is that film exposure to bacteria led to a decrease in RMS roughness. For example, the RMS for $PAC_{18}Na/PDADMAC$ decreased from 1.42 (Figure 7$a_0$) to 0.975 nm (Figure 7$a_2$), which further resulted in a similar contact angle. The increase of the alkyl chain of the cationic surfactant led to the formation of random superficial particles (ball-like) on top of the film for $(PAC_{18}Na\text{-}C_{14}TAB\text{-}NaCl/PDAMAC)_{20}$ and $(PAC_{18}Na\text{-}C_{18}TAB\text{-}NaCl/PDAMAC)_{20}$ polyelectrolyte–surfactant multilayers [18].

After AFM mapping, the data showed that the morphology of the investigated films in the presence of *P. aeruginosa* presented changes in film structure. All films presented a morphology consisting of hills and valleys, which, according to the line scans (vertical direction), were located within 4 nm for the $PAC_{18}Na/PDADMAC$ (Figure 7$a_3$) film and 11 nm for the $PAC_{18}Na\text{-}C_{14}TAB/PDADMAC$ (Figure 7$b_2$) film, respectively. Moreover, the presence of *P. aeruginosa* ATCC 27853 in the aqueous media caused holes in the surface for $(PAC_{18}Na/PDADMAC)_{20}$ and $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$ films. The RMS roughness was 0.914 for $(PAC_{18}Na/PDADMAC)_{20}$ (Figure 7$a_3$) and 1.275 for $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$ (Figure 7$b_3$), while for $(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$ (Figure 7$c_3$), the RMS was 0.864. The water contact angle data for hydrophobically modified poly(acrylate) and their complexes with cationic surfactants showed a decrease in contact angle values for the films treated with *P. aeruginosa* ATCC 2785. For example, the contact angle for untreated $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$ was 107°, while for the exposed one, the contact angle was 98°. For $(PAC_{18}Na\text{-}C_{18}TAB/PDADMAC)_{20}$ exposed to the bacteria with *S. aureus*, the contact angle was calculated to be 113°, and for the uninfected one, the CA was 124°.

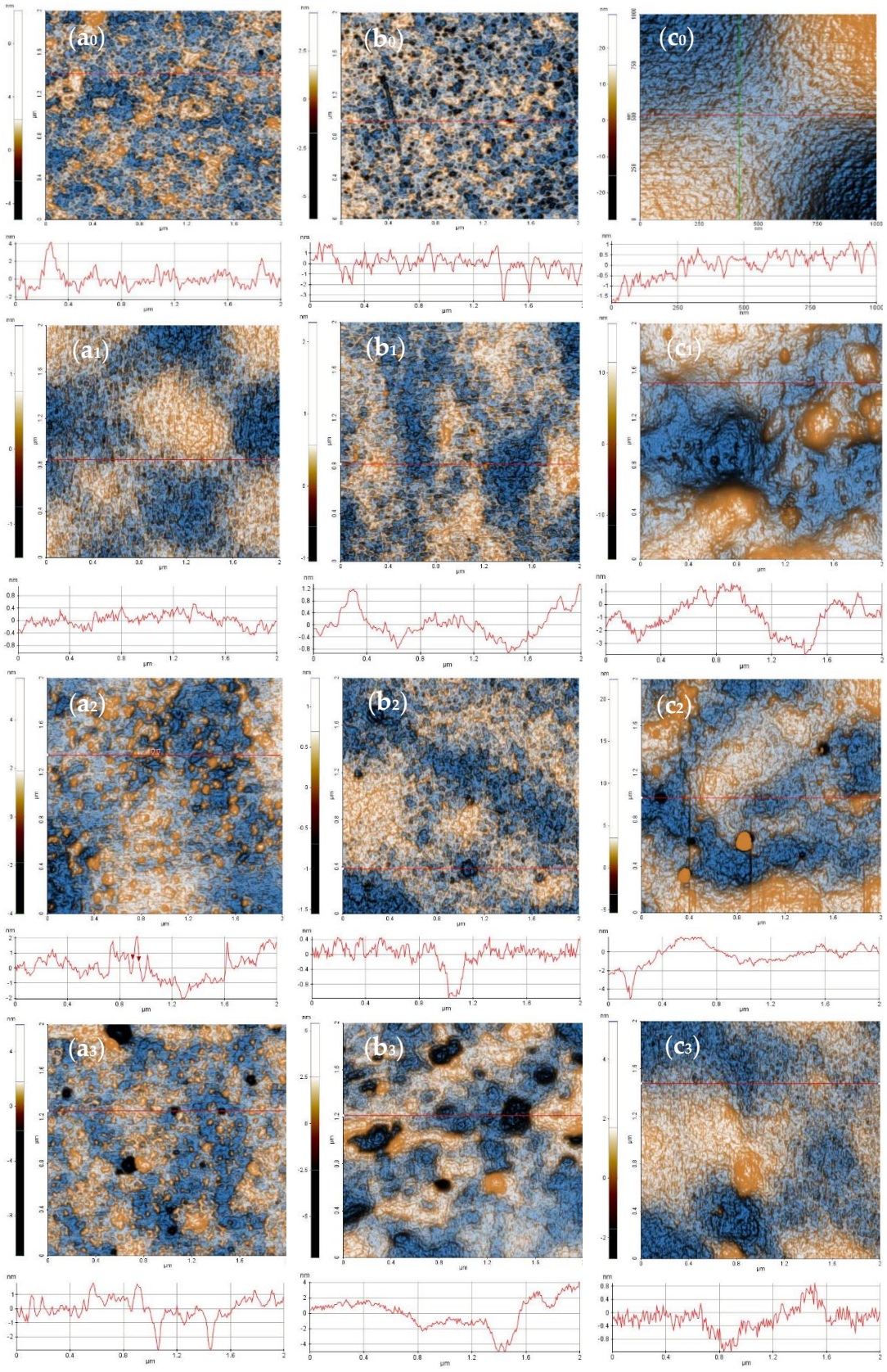


**Figure 7.** Two-dimensional topographic atomic force microscopy (AFM) images of multilayer films of (**a**) $(PAC_{18}Na/PDADMAC)_{20}$, (**b**) $(PAC_{18}Na\text{-}C_{14}TAB/PDADMAC)_{20}$, and (**c**) $(PAC_{18}Na\text{-}C_{18}TAB/PDADMAC)_{20}$ exposed to *E. coli* ATCC 25922 (subscript 1), *S. aureus* ATCC 25923 (subscript 2), *P. aeruginosa* (subscript 3), and without bacteria (subscript 0). Characteristic surface profiles (line scans) are shown near each AFM image. The subscript 20 indicates the number of bilayers.

In Figure 8, two-dimensional topographic AFM images at the scale of $2 \times 2$ μm$^2$ and the water contact angle profiles of the polyelectrolyte or polyelectrolyte–surfactant multilayered films with *C. albicans*, together with the characteristic surface profiles (line scans) along the horizontal direction, are presented.

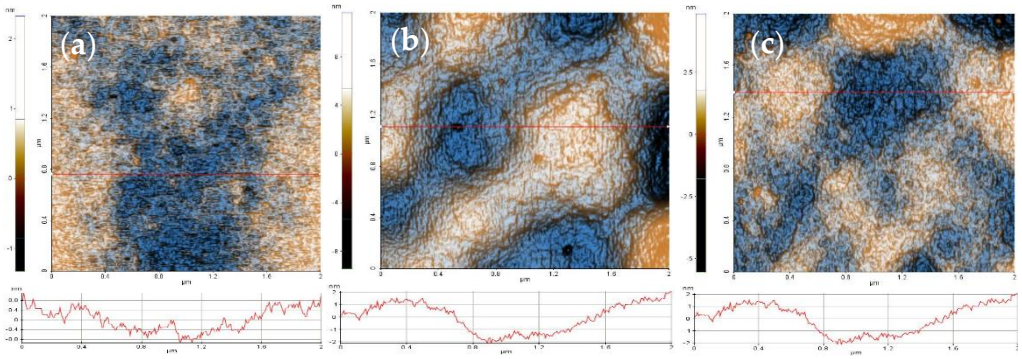


**Figure 8.** The AFM images and water drop contact angle profiles for: (**a**) $(PAC_{18}Na/PDADMAC)_{20}$, (**b**) $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, and (**c**) $(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$ multilayer films exposed to *C. albicans* ATCC 10231 fungus. Characteristic surface profiles (line scans) are shown beneath each image.

The multilayered films presented a morphology consisting of hills and valleys, which, according to the line scans (vertical direction), were located within 1.2 nm for the $PAC_{18}Na/PDADMAC$ film and 13 nm for the $PAC_{18}Na\text{-}C_{14}TAB/PDADMAC$ film, respectively. The RMS roughness was 3.649 for $(PAC_{18}Na/PDADMAC)_{20}$ and 2.731 for $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$, while for $(PAC_{18}Na\text{-}C_{18}TAB/PDAMAC)_{20}$, the RMS roughness was 10.407.

## 4. Conclusions

Hydrophobically modified polyacrylate and their complexes with cationic surfactants have demonstrated their abilities in multilayer film fabrication via electrostatic layer-by-layer assembly. Our study revealed that $(PAC_{18}Na/PDADMAC)_{20}$ and $(PAC_{18}Na\text{-}C_{14}TAB/PDAMAC)_{20}$ systems inhibited the growth of the *S. aureus* by 100%. However, the mentioned films showed poor activity towards *C. albicans*. Future studies will develop complex films to further increase the antimicrobial nature of multilayered structured films. Thus, the use of hydrophobically modified PAA polymer–cationic surfactant complexes proved to be an effective way to construct thin films with remarkable stability, broad antibacterial activity, and low cytotoxicity. These films could find applications in self-cleaning and/or antimicrobial coatings for biomedical devices or decontamination of public areas.

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