Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2023, **33**, 562–564

Mendeleev Communications

Do cationic polymer coatings retain their biocidal activity after washing with water?

Irina G. Panova,*[a] Anastasiya Yu. Lokova,[a] Dmitry V. Bagrov,[b] Nataliya G. Loiko,[c] Yuriy A. Nikolaev[c] and Alexander A. Yaroslavov[a]

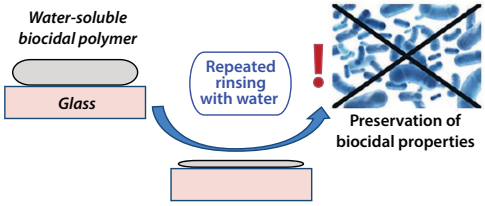
[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. Fax: +7 495 939 0174; e-mail: igpan@mail.ru*

[b] *Department of Biology, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation*

[c] *Federal Research Centre 'Fundamentals of Biotechnology' of the Russian Academy of Sciences, 119071 Moscow, Russian Federation*

DOI: 10.1016/j.mencom.2023.06.039

Aqueous solutions of poly(diallyldimethylammonium chloride) and its electrostatic complexes with sodium polyacrylate were deposited onto glass surfaces. Upon successive washing cycles, they formed thin stable coatings that exhibited antimicrobial activity towards gram-positive and gram-negative bacteria. The obtained results are valuable for the development of antibacterial coatings.



Keywords: polycation, polyanion, polycomplexes, polymeric coating, removal with water, antimicrobial activity.

Surfaces of materials around us are contaminated with various microorganisms, and a significant part of them is classified as pathogenic and should be inactivated.[1–3] Polymers with cationic groups (polycations) have proven to be good biocidal agents.[3–5] It has been shown using native cells and model bilayer lipid vesicles (liposomes) that polycations dissolved in water are capable to bind to cells and induce deep changes in their membranes, finally resulting in the disruption and death of cells.[6–8] Being immobilized on the surface, polycations adsorb cells from a surrounding solution and inactivate them,[8–10] thus suppressing biofilm formation.[10] Polycationic coatings, which can additionally contain low molecular weight biocides or nanosized biocidal constructs,[7] appeared to be an effective protection for various materials.

Polycationic coating is typically formed *via* deposition of an aqueous polymer solution onto the surface to be modified with subsequent drying.[4,11] This simple technique is also environmentally friendly since water is used to make the formulation. However, it should be noted that the coatings are often used in damp environment or subjected to wet cleaning, which can affect their quality. Conventional ionic polymers (polyelectrolytes, PEs) are easily dissolved in water;[4,12] therefore, the polymer can be removed from the surface, resulting in loss of its antimicrobial properties.[13]

Additional stabilization of biocidal coatings can be achieved by hydrophobic modification of cationic PEs: either covalent attachment of hydrophobic fragments ('true' hydrophobicity),[14] or complexation with special, usually water-insoluble, substances ('induced' hydrophobicity).[15] The hydrophobization makes coatings less sensitive to water,[14,15] which allows one to expect their preservation or only partial removal after repeated washings. In any case, the question remains as to whether the repeatedly washed polymer films exhibit the antimicrobial activity and, in general, the composition of the coatings and their biocidal properties are related.

In the present article we compare the stability of cationic polymer coatings formed by a conventional cationic polymer, poly(diallyldimethylammonium chloride) (PDADMAC), and its interpolycomplexes (IPCs), with an anionic polymer, sodium polyacrylate (PANa), to repeated washings with water.[16,17] A partial neutralization of PDADMAC charges with opposite PANa charges rendered the polycomplexes an overall positive charge while the fragments with mutually neutralized charges in the IPCs acted as hydrophobic blocks,[18–20] which was expected to stabilize the IPC coatings according to the induced hydrophobicity mechanism described above. In parallel, we evaluated the antimicrobial properties of the finally washed IPC coatings and showed that their activity is only slightly lower than that of the initial unwashed coatings. This observation has not been previously described or discussed.

The three aqueous formulations were used for the coating fabrication: a solution of PDADMAC and two solutions of IPC with the cationic PDADMAC groups to anionic PANa groups at a molar ratio $Z = [+]/[-] = 0.2$ (IPC-0.2) and 0.4 (IPC-0.4). An aliquot of each formulation was deposited onto a glass slide to cover the entire surface. The samples were dried to a constant weight, which was recalculated to the initial weight of the dried polymer film (m_{orig}). Then, double distilled water was applied to the coated glass. The sample was dried again 2 min after the water removal and the weight of remained polymer film (m_{rem}) was determined (see details of the experimental procedure in Online Supplementary Materials). For each polymer coating, six successive washing–drying cycles were done. The results are shown in Figure 1 as a percentage of the polymer remained on the glass ($m_{rem}/m_{orig} \times 100\%$) *vs.* the number of washing–drying cycles.

The following three points are noteworthy. First, each coating has lost its weight during the washing procedure. At each stage, the PDADMAC coating showed the greatest loss. Second, the complexation of PDADMAC with PANa reduced an ongoing

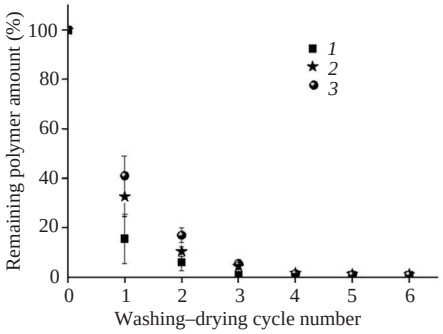


Figure 1 Percentage of the polymer remained on the glass *vs.* the number of washing–drying cycles: (*1*) PDADMAC, (*2*) IPC-0.2 and (*3*) IPC-0.4.

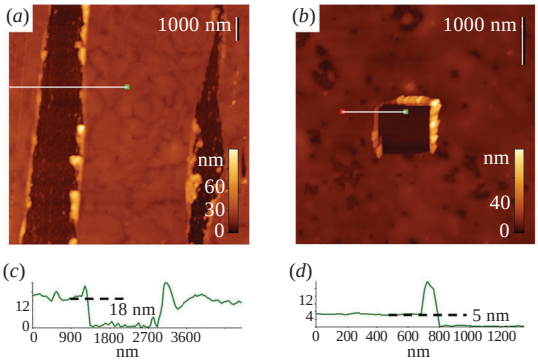


Figure 3 AFM images of 6 times washed (*a*) PDADMAC and (*b*) IPC-0.4 polymer coatings; the section profiles along the white lines in (*c*) PDADMAC and (*d*) IPC-0.4 samples.

weight loss. Third, less than 1% polymers left on the glass surface after 4 washing–drying cycles. Thus, all the three coatings from cationic polymer formulations were quickly removed from the surface by water treatment. In other words, modification of cationic PDADMAC with anionic PANa did not affect the number of washing cycles that provided the maximum weight loss of the coating.

As follows from the published results,[21–24] the electrostatic complexation of cationic polymers with anionic polymer microspheres, bio-colloids and planar objects results in the formation of interface polymer layers, which retain on the surface in concentrated salt solutions. Therefore, it was reasonable to expect the existence of a residual polycationic layer on the glass surface even after 4 washing–drying cycles, and this layer should most likely demonstrate antimicrobial properties.

The morphology of polymer coatings before and after washing with water was established using scanning electron microscopy (SEM). The crystals of low molecular weight salts, which came from the buffer solution, can be seen on the surface of the initial PDADMAC coating shown in Figure 2(*a*). As for the IPC coatings [Figure 2(*c*)], the number of crystals is greater due to a release of small counterions originally bound to cationic and anionic polymer chains. Two consecutive washings of the coatings with double distilled water leads to a complete removal of salts [Figure 2(*b*),(*d*)]; the double-washed coatings are characterized by the uniform surface without microstructural defects.

The thickness of the polymer coatings was estimated using atomic force microscopy (AFM). Prior to measurements, the coatings were treated 6 times with double distilled water to ensure complete removal of low-molecular weight salts and leave the minimal residual polymer layer on glass. The AFM images were obtained in semi-contact mode. To quantify the coating thickness, the PDADMAC sample was scratched using a wooden tip, and the IPC sample was scratched using the AFM tip, scanning a small region in contact mode.[25] Typical AFM images of the scratched PDADMAC and IPC-0.4 surfaces are shown in Figures 3(*a*) and 3(*b*), respectively. The thickness of the coating, measured by sections as shown in Figure 3(*c*),(*d*), was 18 ± 2 nm for PDADMAC and 5.5 ± 1.5 nm for IPC-0.4. This difference probably resulted from the structure of the IPC-0.4 in which there was a hydrophobic block from mutually neutralized cationic and anionic groups of both PEs. This led to additional attraction between the IPC particles forming a denser but thinner film after drying.

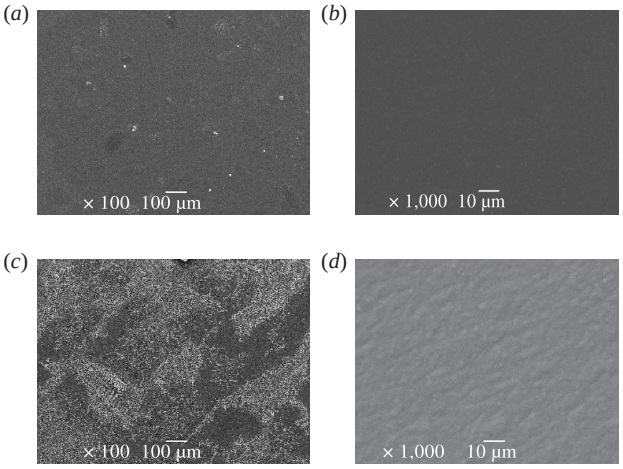


Figure 2 SEM micrographs of the polymer coatings of (*a*) PDADMAC on glass before and (*c*) after two washings with water; (*b*) IPC-0.4 on glass before and (*d*) after two washings with water.

The IPC-0.4 coating had a porous structure [Figure 3(*b*)] with a typical pore size of 200 nm or less. The pore depth coincides with the thickness of the IPC-0.4 coating, estimated from the height profiles [Figure 3(*d*)]. This indicates that the pores penetrate the entire thickness of the IPC-0.4 coating.

It is natural to assume that drying of the IPC solution caused the appearance of low molecular weight salts not only on the surface of the coating, but also inside it. The water dissolved the salt on the coating surface and washed it out from the bulk of the polymer coating that resulted in continuous pore formation as shown in Figure 3(*b*). The dried PDADMAC coating contained a lower amount of salt that gave a less pronounced pore-forming effect after treatment of the coating with water.

Antimicrobial activity of polymer coatings was tested with gram-negative bacteria *Pseudomonas aeruginosa* 4.8.1 and gram-positive bacteria *Staphylococcus aureus* 209P from the microorganism collection of the Federal Research Centre 'Fundamentals of Biotechnology', RAS. The cationic coatings from PDADMAC, IPC-0.2 and IPC-0.4 were pre-washed 6 times and dried.

An aliquot with 200–800 bacterial cells was applied to the coatings, 15 min after the cells were washed with distilled water onto the agar substrate, where a colony was formed from each survived bacterial cell within 2 days.[26] After counting the grown colonies, the percentage of survived cells compared to the initial number of cells was calculated (Table 1).

Table 1 Percentage of survived cells *vs.* their content in an applied aliquot after 15 min incubation on the polymer coatings.

Polymeric formulation	Percentage of survived cells					
	P. aeruginosa			*S. aureus*		
	Number of cells in applied aliquot					
	200 ± 13	600 ± 41	800 ± 48	200 ± 12	600 ± 37	800 ± 49
PDADMAC	19.0	34.4	67.1	1.0	4.9	7.4
IPC-0.2	6.2	26.0	46.4	0	1.2	1.6
IPC-0.4	25.9	58.9	75.9	4.4	15.2	20.6

Table 2 Percentage of survived *P. aeruginosa* cells after 15 and 30 min incubation on the polymer coatings. An aliquot with 600 ± 40 cells was used.

Polymeric formulation	Percentage of survived *P. aeruginosa* cells	
	15 min incubation	30 min incubation
PDADMAC	34.4	3.7
IPC-0.2	26.0	3.1
IPC-0.4	58.9	40.2

As follows from Table 1, the antimicrobial effect depends on the number of cells in the applied aliquot, that is, the density of cells on the polymer coating. The more cells were in the applied aliquot, the less the biocidal effect of the coating was manifested. Gram-positive *S. aureus* cells were sensitive to all cationic polymeric coatings: for a 200-cell aliquot, the percentage of survived cells did not exceed 5%. *P. aeruginosa* cells showed greater stability (survival) on the polymeric coatings.

The IPC-0.2 coating was the most biocidal of the three tested formulations. After a 15-min incubation, the percentage of survived *S. aureus* cells ranged from 0 to 1.6% and that of *P. aeruginosa* ranged from 6.2 to 46.4%. In this regard, preliminary considerations can be made. The biocide properties of IPCs were due to their cationic groups and mutually neutralized hydrophobic blocks. Both factors ensure IPC binding to bacteria and induce reorganization in the bacterial membranes followed by the disruption and death of cells. The IPC-0.2 showed the greatest antimicrobial activity that may indicate an optimal combination of cationic and hydrophobic fragments in the IPC structure. However, this assumption, based on limited experimental material, needs further confirmation.

A rise in the contact time of cells with the coating (incubation time) expectedly reduced their survival. With increasing contact time from 15 to 30 min, the percentage of survived *P. aeruginosa* cells decreased by 1.5–10 times (Table 2). The cationic coatings from PDADMAC and IPC-0.2 showed the best results with an 8–10-fold decrease in the cell survival.

In summary, deposition of aqueous solutions of cationic PDADMAC and cationic IPCs onto the glass slides and further drying resulted in the polymeric coatings, which can be removed from the surface *via* water treatment. A few successive washing cycles were sufficient to wash out the majority of polymers. Complexation of PDADMAC with anionic PANa and formation of IPCs with an excess of polycationic units does not improve the stability of polymer coatings to water. The thin polymer layers (less than 20 nm in thickness), which remained on glass after 6 washing–drying cycles, show high antimicrobial activity towards gram-positive and gram-negative bacteria with the best results for PDADMAC and IPC-0.2.

The work was supported by the Russian Science Foundation (project no. 22-13-00124).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2023.06.039.

References

1 D. Olmos and J. González-Benito, *Polymers*, 2021, **13**, 613.
2 B. Balasubramaniam, Prateek, S. Ranjan, M. Saraf, P. Kar, S. P. Singh, V. K. Thakur, A. Singh and R. K. Gupta, *ACS Pharmacol. Transl. Sci.*, 2021, **4**, 8.
3 M. M. Konai, B. Bhattacharjee, S. Ghosh and J. Haldar, *Biomacromolecules*, 2018, **19**, 1888.
4 Y. Xue, H. Xiao and Y. Zhang, *Int. J. Mol. Sci.*, 2015, **16**, 3626.
5 E. L. Meier and Y. Jang, *Curr. Opin. Biomed. Eng.*, 2023, **26**, 100448.
6 N. O. Kozlova, I. B. Bruskovskaya, I. B. Okuneva, N. S. Melik-Nubarov, A. A. Yaroslavov, V. A. Kabanov and F. M. Menger, *Biochim. Biophys. Acta, Biomembr.*, 2001, **1514**, 139.
7 V. M. Misin, A. A. Zezin, D. I. Klimov, A. V. Sybachin and A. A. Yaroslavov, *Polym. Sci., Ser. B*, 2021, **63**, 459.
8 L. Timofeeva and N. Kleshcheva, *Appl. Microbiol. Biotechnol.*, 2011, **89**, 475.
9 M. Bagheri, M. Beyermann and M. Dathe, *Bioconjugate Chem.*, 2012, **23**, 66.
10 J. Hoque, S. Ghosh, K. Paramanandham and J. Haldar, *ACS Appl. Mater. Interfaces*, 2019, **11**, 39150.
11 J. Song and J. Jang, *Adv. Colloid Interface Sci.*, 2014, **203**, 37.
12 A. Rabiee, A. Ershad-Langroudi and M. E. Zeynali, *Rev. Chem. Eng.*, 2015, **31**, 239.
13 B. Song, E. Zhang, X. Han, H. Zhu, Y. Shi and Z. Cao, *ACS Appl. Mater. Interfaces*, 2020, **12**, 21330.
14 A. R. Biery and D. M. Knauss, *J. Polym. Sci.*, 2023, **61**, 197.
15 H. Yu, L. Liu, H. Yang, R. Zhou, C. Che, X. Li, C. Li, S. Luan, J. Yin and H. Shi, *ACS Appl. Mater. Interfaces*, 2018, **10**, 39257.
16 I. G. Panova, A. V. Sybachin, V. V. Spiridonov, K. Kydralieva, S. Jorobekova, A. B. Zezin and A. A. Yaroslavov, *Geoderma*, 2017, **307**, 91.
17 O. A. Novoskoltseva, E. V. Chernikova, V. B. Rogacheva and A. B. Zezin, *Polym. Sci., Ser. B*, 2015, **57**, 132.
18 V. A. Izumrudov, *Russ. Chem. Rev.*, 2008, **77**, 381 (*Usp. Khim.*, 2008, **77**, 401).
19 H. Dautzenberg and N. Karibyants, *Macromol. Chem. Phys.*, 1999, **200**, 118.
20 V. A. Kabanov, *Russ. Chem. Rev.*, 2005, **74**, 3 (*Usp. Khim.*, 2005, **74**, 5).
21 A. S. Malinin, I. V. Kalashnikova, A. A. Rakhnyanskaya and A. A. Yaroslavov, *Polym. Sci., Ser. A*, 2012, **54**, 81.
22 O. V. Ivashkov, A. V. Sybachin, A. A. Efimova, D. V. Pergushov, V. N. Orlov, H. Schmalz and A. A. Yaroslavov, *ChemPhysChem*, 2015, **16**, 2849.
23 I. Szilagyi, G. Trefalt, A. Tiraferri, P. Maroni and M. Borkovec, *Soft Matter*, 2014, **10**, 2479.
24 A. A. Rakhnyanskaya, I. D. Pebalk, V. N. Orlov, I. A. Gritskova, N. I. Prokopov and A. A. Yaroslavov, *Polym. Sci., Ser. A*, 2010, **52**, 483.
25 O. V. Morozova, O. A. Levchenko, Z. A. Cherpakova, V. V. Prokhorov, N. A. Barinov, E. A. Obraztsova, A. M. Belova, K. A. Prusakov, K. G. Aldarov, D. V. Basmanov, V. N. Lavrenova, E. R. Pavlova, D. V. Bagrov, V. N. Lazarev and D. V. Klinov, *Int. J. Adhes. Adhes.*, 2019, **92**, 125.
26 I. G. Panova, E. A. Shevaleva, I. A. Gritskova, N. G. Loiko, Y. A. Nikolaev, O. A. Novoskoltseva and A. A. Yaroslavov, *Polymers*, 2022, **14**, 4598.

Received: 21st February 2023; Com. 23/7107