Available online at www.sciencedirect.com

ScienceDirect

Mendeleev Commun., 2024, **34**, 369–371

Mendeleev Communications

Polycomplexes to modulate bactericidal activity of cetylpyridinium bromide

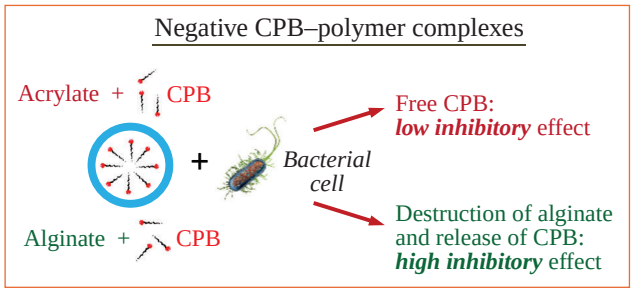
Olga A. Novoskoltseva,*[a] Darya G. Sinelnikova,[a] Nataliya G. Loiko,[b] Yury A. Nikolaev[b] and Alexander A. Yaroslavov[a]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: nsn07@yandex.ru*

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

DOI: 10.1016/j.mencom.2024.04.018

Cetylpyridinium bromide, a quaternary ammonium surfactant, was electrostatically complexed with an excess of anionic polymers such as biodegradable sodium alginate or non-biodegradable sodium polyacrylate. The binding of surfactant with alginate delivered polymer–surfactant complexes capable of killing both more surfactant-sensitive *Escherichia coli* and less surfactant-sensitive *Pseudomonas aeruginosa* bacteria, while the binding with acrylate gave complexes active only against *Escherichia coli*. The results obtained show a way for adjustment of the antimicrobial formulation to a specific pathogen to be destroyed.



Keywords: polymer–surfactant complexes, quaternary ammonium compounds, pyridinium salts, biodegradable polymers, bactericides, gram-negative bacteria, minimum inhibitory concentration.

Pathogenic microorganisms are one of the leading causes of mortality initiating severe infectious diseases such as pneumonia, sepsis, surgical infections, *etc*.[1] Among pathogenic microorganisms, gram-negative bacteria are most active in the community and hospital settings. Gram-negative bacteria are more resistant to antimicrobial agents in comparison to gram-positive bacteria; they spread faster and pose a greater danger to humans.[2] In recent decades, there has been significant concern about the rise in antibiotic-resistant bacteria, with gram-negative species making the main contribution to this problem.[2–5] All these factors dictate an urgent need for efficient biocides, especially affecting resistant gram-negative microorganisms.[6–8]

Quaternary ammonium compounds (QACs) are widely used as antiseptics and disinfectants whose practical application has been grown tremendously during the COVID-19 pandemic.[9,10] They are also applied for drug and gene delivery and protein folding.[10] In aqueous solutions, cationic micelle-forming QACs interact with anionic polymers leading to new polymer–surfactant complexes (PSCs).[11–21] The latter are stabilized by hydrophobic interactions of the surfactant aliphatic tails, which ensures micelle formation and multiple ionic contacts between oppositely charged groups of micelles and polymer chains.[11–15] The complexation allows one to concentrate the biocidal QAC in a small volume; the use of a biodegradable polyanion makes PSCs sensitive to pathogenic microbes. Acting together, these factors can increase the therapeutic effect of QAC incorporated in the polyanion matrix.

In the current article, we describe complexation of *N*-cetylpyridinium bromide (CPB), a cationic surfactant, with sodium alginate (ALG), a native polysaccharide, and studies on the toxicity of the CPB–ALG complex to gram-negative bacteria. We compare the behavior of the CPB–ALG complex with the behavior of complex composed of CPB and non-biodegradable anionic polymer such as synthetic sodium polyacrylate (PANa), and discuss a possible mechanism of the toxic effect of both complexes towards gram-negative bacterial cells.

Binding of CPB to the polyanions was performed *via* addition of a CPB solution in 0.01 M Tris buffer with pH 7 to an anionic polymer solution in the same buffer. Cationic CPB (Sigma-Aldrich) and anionic polymers ALG (ISP, UK) and PANa with M_w = 100 kDa (Sigma-Aldrich) were used. Molar concentration of CPB was measured spectrophotometrically taking experimentally determined molar extinction coefficient of 4.100 dm^3 mol^{-1} cm^{-1} at λ = 259 nm. Concentrations of ALG and PANa were measured based on potentiometric titration of ALG/PANa aqueous solutions and expressed as the number of moles of anionic COOH groups per liter.

On addition of a CPB solution to a solution of ALG or PANa, the mixtures became progressively turbid (Figure 1) due to formation of CPB–ALG and CPB–PANa PSC particles. In both cases, the maximum turbidity is observed at molar ratios of cationic CPB and anionic ALG/PANa units Z = [CPB]/[ALG] = = [CPB]/[PANa] close to 1, which indicates complete neutralization of the negative polyanion charges by cationic surfactant charges. This, in turn, means that the compositions of the CPB–polymer complexes are identical to the compositions of the corresponding CPB–polymer mixtures.

In the range $Z \leqslant 0.7$ in Figure 1, the turbidity did not exceed 20% that allowed the PSC particles with the hydrodynamic diameter of 90–300 nm that was measured with dynamic light scattering to occur. Aggregative stability of these particles was due to their negative charges provided by an excess of anionic polymer.

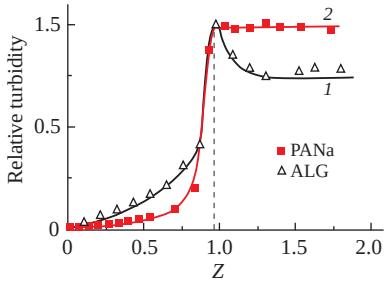


Figure 1 Dependence of the relative turbidity of the (*1*) CPB–ALG and (*2*) CPB–PANa mixtures on the *Z* ratio at 500 nm. 0.01 M Tris buffer, pH 7; [PANa] = 10^{-3} M; [ALG] = 4.5×10^{-4} M.

The colloidally stable PSC formulations with $Z \leqslant 0.7$ were used in antimicrobial experiments. Inhibitory activity of the formulations was quantitatively characterized by their minimum inhibitory concentration (MIC), the lowest concentration which inhibited the growth of microorganisms in solution.[22,23] Non-pathogenic strains of gram-negative encapsulated motile bacteria were used as test cultures: *Escherichia coli* MG 1655 K12, key model prokaryotic organisms for microbiological testing, and more resistant *Pseudomonas aeruginosa* 4.8.1, one of the major pathogens in nosocomial infections.[24–26] The bacterial cultures were from the microorganism collection of the FRC Biotechnology RAS.

MIC values for CPB–ALG and CPB–PANa polycomplexes with $Z = 0.1$, 0.3, 0.5 and 0.7 together with MIC values for CPB and individual polyanions against both bacteria are shown in Figures 2 and 3. As expected, ALG and PANa showed no toxicity to the bacterial cells up to the maximal tested polymer concentration of 30000 μM, whereas CPB had a strong bactericidal effect with a MIC value of 14 μM for the cells of *E. coli* (see Figure 2) and 67 μM for *P. aeruginosa* (see Figure 3).

All complexes showed an antimicrobial effect on *E. coli* (see Figure 2), while their inhibitory activity was within 14 ± 2 μM, *i.e.* at a level of individual CPB surfactant, and did not depend on the type of the polymer: biodegradable or not. The same PSC formulations demonstrated a more complicated antimicrobial effect being added to *P. aeruginosa* bacteria (see Figure 3). The CPB–ALG formulations were nearly as active as individual CPB surfactant (100 ± 10 μM); such behavior was similar to that of CPB–ALG formulations towards *E. coli* bacteria. Contrastingly, the activity of CPB–PANa formulations decreased sharply towards the activity of individual ALG. In other words, the complexation of CPB with biodegradable ALG did not change the activity of CPB to *E. coli* and *P. aeruginosa*. The complexation of CPB with non-biodegradable PANa also did not affect the activity of surfactant to *E. coli* but dramatically reduced its activity to *P. aeruginosa*.

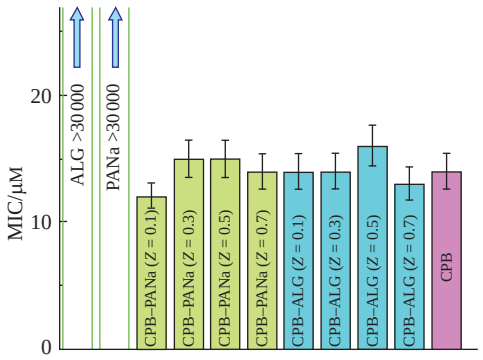


Figure 2 MIC values for ALG, PANa, CPB and CPB–ALG and CPB–PANa complexes against *E. coli*. Standard deviations of 10% are shown as error bars.

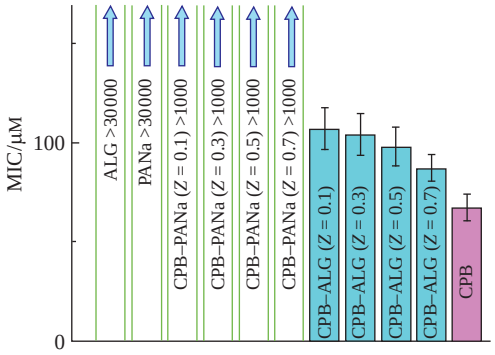


Figure 3 MIC values for ALG, PANa, CPB and CPB–ALG and CPB–PANa complexes against *P. aeruginosa*. Standard deviations of 10% are shown as error bars.

In order to clarify a possible mechanism of CPB–polymer formulations to bacterial cells, we turned to the literature. Complexation of CPB to anionic polymers has been intensively investigated earlier.[11,14,16] In has been found in particular that the complexation was a highly cooperative process which occurs in a narrow range of CPB concentration in solution. The equilibrium concentrations of free CPB (unbound to ALG[14,16] and PANa[11]) lie in the vicinity of 10 μM. This value is comparable with MIC for individual CPB against *E. coli* bacteria. If so, the concentration of free CPB is sufficient to kill *E. coli* as it follows from the data in Figure 2. Both biodegradable CPB–ALG and non-biodegradable CPB–PANa complexes reveal the same activity against *E. coli*, and this activity is close to that of individual CPB. No need in additional CPB, initially incorporated into the PSC, is required to kill *E. coli*.

As regards the activity of CPB–polymer complexes to *P. aeruginosa* (see Figure 3), cells of the latter are more resistant to CPB resulting in a higher MIC value of 67 against 14 μM for *E. coli*. The MIC value of 67 μM exceeds that for free CPB concentration in solutions of the CPB–polymer complexes equal to 10 μM (see above). For this reason, it seems that the CPB–polymer complexes would not kill bacteria. This is actually true for the non-biodegradable CPB–PANa complexes whose MIC values are many times higher than those for individual CPB indicating an extremely low toxicity of CPB–PANa for *P. aeruginosa*. At the same time, the biodegradable CPB–ALG complexes kill *P. aeruginosa*. Obviously, this happens because *P. aeruginosa* bacteria can degrade polysaccharide[27–30] and finally meet CPB which kills them.

Summarizing, negatively charged polymer–colloid complexes were prepared *via* binding of quaternary pyridinium compound such as CPB to an excess of anionic polymers such as native polysaccharide ALG and synthetic PANa. The biodegradable CPB–ALG complexes are active against both more CPB-sensitive *E. coli* and less CPB-sensitive *P. aeruginosa* bacteria, while the non-biodegradable CPB–PANa complexes are active only against *E. coli* and inert to *P. aeruginosa*. Antimicrobial action of polycomplexes is modulated by different availability of anionic polymer for bacterial enzymes and different sensitivity of bacteria to biocidal CPB. This demonstrates a means of adjusting the antimicrobial formulation for the specific pathogen to be destroyed.

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

References

1 X. Zhu, Y. Li, X. Zhang, P. Zhang, Q. Tian, C. Ma and C. Shi, *Curr. Microbiol.*, 2023, **80**, 97.
2 S. Alfei and A. M. Schito, *Polymers*, 2020, **12**, 1195.

3 V. Nagvekar, S. Sawant and S. Amey, *J. Global Antimicrob. Resist.*, 2020, **22**, 457.
4 C. Overly Cottom, R. Stephenson, L. Wilson and N. Noinaj, *Antibiotics*, 2023, **12**, 679.
5 A. N. Vereshchagin, N. A. Frolov, V. Yu. Konyuhova, K. A. Hansford and M. P. Egorov, *Mendeleev Commun.*, 2019, **29**, 523.
6 R. R. Davletshin, A. M. Gayneev, E. A. Ermakova, N. V. Davletshina, I. V. Galkina, K. A. Ivshin, M. P. Shulaeva and O. K. Pozdeev, *Mendeleev Commun.*, 2022, **32**, 180.
7 N. V. Davletshina, E. A. Ermakova, D. R. Dolgova, R. R. Davletshin, M. P. Shulaeva, I. I. Stoikov, B. I. Gareev and R. A. Cherkasov, *Mendeleev Commun.*, 2023, **33**, 627.
8 D. G. Sinelnikova, O. A. Novoskoltseva, N. G. Loiko, Yu. A. Nikolaev and A. A. Yaroslavov, *Mendeleev Commun.*, 2024, **34**, 31.
9 J. M. Boyce, *Antimicrob. Resist. Infect. Control*, 2023, **12**, 32.
10 A. M. Carmona-Ribeiro and L. D. de Melo Carrasco, *Int. J. Mol. Sci.*, 2013, **14**, 9906.
11 V. A. Kabanov, A. B. Zezin, V. B. Rogacheva, Y. V. Khandurina and O. A. Novoskoltseva, *Macromol. Symp.*, 1997, **126**, 79.
12 I. Satake, T. Takahashi, K. Hayakawa, T. Maeda and M. Aoyagi, *Bull. Chem. Soc. Jpn.*, 1990, **63**, 926.
13 P. S. Kuhn, Y. Levin and M. C. Barbosa, *Chem. Phys. Lett.*, 1998, **298**, 51.
14 A. Malovikova, K. Hayakawa and J. C. Kwak, *ACS Symp. Ser.*, 1984, **253**, 225.
15 N. Khan and B. Brettmann, *Polymers*, 2018, **11**, 51.
16 J. Mirtič, K. Kogej, S. Baumgartner, G. Smistad, J. Kristl and M. Hiorth, *Int. J. Pharm.*, 2016, **511**, 774.
17 A. Mezei and R. Mészáros, *Langmuir*, 2006, **22**, 7148.
18 S. Guillot, D. McLoughlin, N. Jain, M. Delsanti and D. Langevin, *J. Phys.: Condens. Matter*, 2003, **15**, S219.
19 J. Courtois and J. F. Berret, *Langmuir*, 2010, **26**, 11750.
20 M. Gradzielski, *Langmuir*, 2022, **38**, 13330.
21 Y. Lapitsky, M. Parikh and E. W. Kaler, *J. Phys. Chem. B*, 2007, **111**, 8379.
22 V. L. Barnes, D. M. Heithoff, S. P. Mahan, J. K. House and M. J. Mahan, *STAR Protoc.*, 2023, **4**, 102512.
23 N. Loiko, Y. Danilova, A. Moiseenko, V. Kovalenko, K. Tereshkina, M. Tutukina, G. El-Registan, O. Sokolova and Y. Krupyanskii, *PLoS One*, 2020, **15**, e0231562.
24 J. A. Lesley and C. D. Waldburger, *J. Biol. Chem.*, 2001, **276**, 30827.
25 D. J. Evans, D. G. Allison, M. R. W. Brown and P. Gilbert, *J. Antimicrob. Chemother.*, 1991, **27**, 177.
26 G. Laverty, S. P. Gorman and B. F. Gilmore, *Pathogens*, 2014, **3**, 596.
27 A. Boyd and A. M. Chakrabarty, *J. Ind. Microbiol.*, 1995, 1**5**, 162.
28 L. Zhang, X. Li, X. Zhang, Y. Li and L. Wang, *Biotechnol. Biofuels*, 2021, **14**, Article 158.
29 H. Ertesvåg, *Front. Microbiol.*, 2015, **6**, 523.
30 F. Thomas, T. Barbeyron, T. Tonon, S. Génicot, M. Czjzek and G. Michel, *Environ. Microbiol.*, 2012, **14**, 2379.

Received: 5th February 2024; Com. 24/7384