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Antimicrobial activity of model microplastics loaded with a toxic polycation

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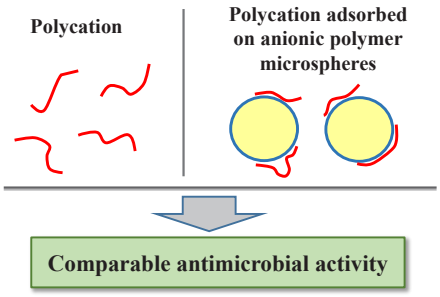
A comparative investigation of the antimicrobial activity of a cationic polymer [poly(diallyldimethylammonium chloride)], model microplastics consisting of anionic butadiene–α-methylstyrene copolymer microspheres, and electrostatic polymer–microsphere complexes was undertaken. The polymer demonstrates a high antimicrobial activity towards gram-positive bacteria *S. aureus* and gram-negative bacteria *E. coli*, the microspheres being practically inert, while the activity of complexes is comparable with the activity of polycation at the same concentration. This means that microplastic particles loaded with toxic substances have a significant negative impact on microorganisms in aquatic environment.



Keywords: microplastics, polycation, anionic polymer microspheres, complexation, antimicrobial activity.

Plastic materials have firmly entered the modern society. The permanently growing production of synthetic plastics has led to a significant increase in the amount of polymer waste and its accumulation in the environment.[1–3] Plastic breaks down into pieces through physical, chemical and biological processes, eventually forming microplastics (MP) 5 mm or smaller in size, which have a negative impact on living organisms, including humans.[4–7] The greatest danger is posed by nano- and microparticles that can penetrate biological barriers.[6–9] In addition, the large specific surface area of such particles is capable of adsorbing huge amounts of toxic compounds and transporting them over long distance.[5,10,11] Toxic compounds may also be contained in plastic particles, because potentially toxic additives (initiators, stabilizers, antioxidants, dyes, *etc*.) are introduced into polymer materials during their production.[3,6,11,12] Due to the variable composition, small size and continuous evolution of plastic particles, they are difficult to work with.[7,11,13,14] Therefore, at present, specially prepared model (engineered) particles are used to study physico-chemical and biological properties of microplastics.[15] This approach allows one to make the reliable composition–structure correlations and predict physiological effects of polymer particles.

Considering the above, the goal of this work was to establish a relationship between the composition of engineered polymer particles loaded with a toxic compound and the total biocidal effect of the resulting complexes. Microspheres (MSs) prepared from the butadiene–α-methylstyrene (7:3) copolymer with carboxylic surface groups were taken as model MP species, which were electrostatically complexed with cationic poly(diallyldimethylammonium chloride) (PDADMAC). This cationic polymer is known to be noticeably toxic to various microorganisms.[16–18] Each PDADMAC macromolecule carries several hundred cationic groups; therefore, it strongly binds to anionic colloidal particles of organic and mineral nature. This property makes it an effective flocculant in water treatment and water purification.[16,19] Being bound to small particles, PDADMAC can leave the treatment plants and spread through water systems over long distances. Cationic polymers bound to particles are able to migrate between them, thus occupying all particles in the system.[20] Redistribution of the polycation may affect the toxicity of the polymer–colloid complex particles.

Microspheres had an average size (hydrodynamic diameter D_h) of 165 nm and electrophoretic mobility (EPM) of –4 μm cm V^{-1} s^{-1}, a parameter associated with their surface charge. PDADMAC had an average molecular mass M_w = 200–350 kDa. Concentration of PDADMAC was expressed in moles of amino groups per liter [N^+]; concentrations of MSs, in moles of carboxylic groups per liter [COOH] (see details in Online Supplementary Materials).

Adsorption of PDADMAC on the MS surface was performed in a 10^{-2} M phosphate buffer solution at pH 7. Addition of a PDADMAC solution to a MS solution resulted in progressive neutralizing the MS surface charges and altering EPM of particles in the system [Figure 1(*a*)]; a complete neutralization of the MS charges was achieved at a charge-to-charge ratio Z = [N^+]/[COOH] = 0.85.

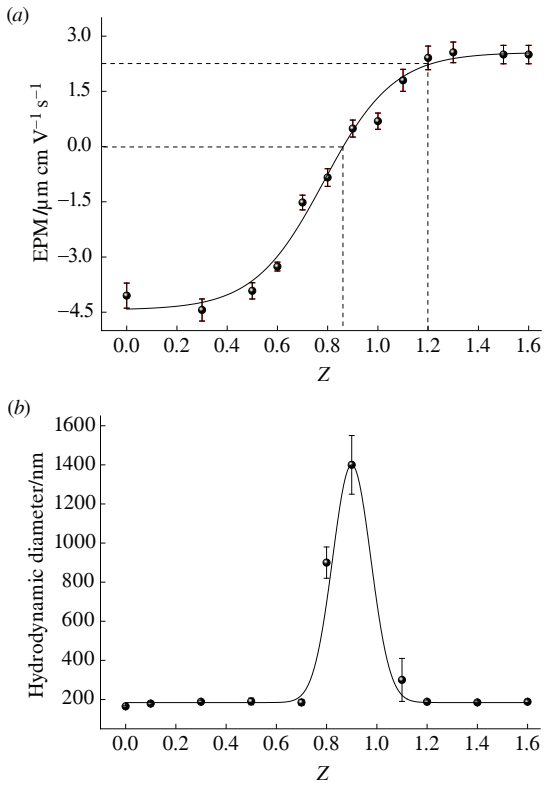


Figure 1 Dependences of (*a*) EPM and (*b*) hydrodynamic diameter of PDADMAC–MS complex on $Z = [N^+]/[COOH]$; MS concentration 4.1×10^{-6} mol dm^{-3}; 10^{-2} M phosphate buffer, pH 7.

The cationic PDADMAC contains quaternary amino groups, all these groups participating in the electrostatic bonding to the anionic MSs. At EPM = 0 the concentration of the positive PDADMAC groups is obviously equal to the concentration of the negative (ionized) MS groups. From here, $Z = [N^+]/[COOH] = 0.85$ means the maximum degree of carboxylic MS groups involved in the electrostatic complexation with PDADMAC. A degree of dissociation of carboxylic MS groups at pH 7 is ~ 0.7.[21] Extra ionized COO^- groups (0.85 – 0.7 = 0.15) appeared due to a cooperative displacement of protons from carboxylic groups by the interaction with PDADMAC.[22,23] An increase in Z over 0.85 led to the formation of positively charged complexes with an excess of adsorbed polycation.

As follows from the published data,[24] the maximum positive EPM value on the EPM *vs.* Z plot corresponds to maximum binding of the polymer to colloidal particles. At higher polymer concentrations, it is detected in solution in a free form, being unbound to particles. In our case, the maximum polymer binding is achieved at $Z = 1.2$ [Figure 1(*a*)].

Measurements of the size of complex particles [Figure 1(*b*)] correlated well with the EPM data, showing the aggregation of the complex particles as their charge neutralized and the stabilization of the complex particles occurred both in the deficiency and the excess of PDADMAC. The stable negative complex particles had a size of 180 ± 8 nm, and the stable positive complex particles had a size of 188 ± 10 nm.

The antimicrobial properties of aqueous polymer formulations were quantified towards gram-positive bacteria *Staphylococcus aureus* 209P and gram-negative bacteria *Escherichia coli* K-12 MG1655 from the collection of the Research Center of Biotechnology, Russian Academy of Sciences, as described elsewhere.[24] Briefly, various aliquots of an 1 wt% polymer solution were added in the glass test tubes with the M9 medium. The tubes were inoculated with the microorganisms and then were placed on a shaker at 28 °C. After two days, the growth of microorganisms was assessed visually; the lowest polymer concentration, where no growth of the cultures in test tubes was observed, was taken as the minimum inhibitory concentration (MIC). In parallel, aliquots of the cultures from the tubes with the polymer concentrations ⩾ MIC were plated on Petri dishes with the LB medium supplemented with agar and the growth of the microorganisms was evaluated. The lowest polymer concentration, where no growth of microorganisms on the dense media was observed, was taken as the minimum bactericidal concentrations (MBC).[24]

The activity of antimicrobial formulations was thus tested in the M9 medium with approx. 0.1 M water–salt solution.[24] At the same time, it is known that electrostatic polymer–colloid complexes dissociate down to the initial components at high salt concentrations.[23,25] Taking this into account, the stability of PDADMAC–MS complexes in aqueous–salt solutions was determined by measuring a relative optical density A of solutions in the presence of increasing NaCl concentrations (a suspension of initial MSs was used as a relative sample). Two complexes were prepared: 'anionic' with an excess of negative MS groups and $Z = 0.8$ and 'cationic' with an excess of positive PDADMAC groups and $Z = 1.2$. In both cases, addition of NaCl led to an increase in the optical density (Figure 2, curves *1* and *2*), which reflected aggregation of particles induced by shielding of the complex particle charges by the charges of small counter-ions Na^+ or Cl^-. The optical density did not alter up to $C_{NaCl} = 0.33$ mol dm^{-3}

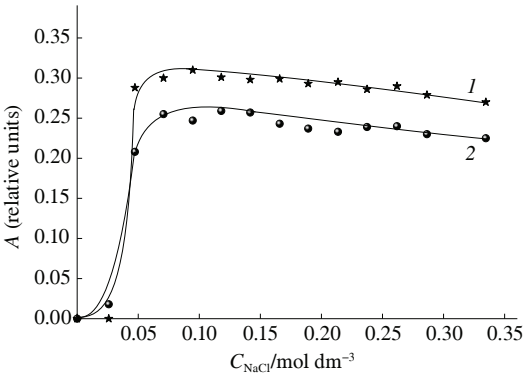


Figure 2 NaCl concentration dependence of the relative optical density A of PDADMAC–MS complexes with (*1*) $Z = 0.8$ and (*2*) $Z = 1.2$ at 500 nm; MSs concentration 4.1×10^{-6} mol dm^{-3}; 10^{-2} M phosphate buffer, pH 7.

Table 1 The MIC and MBC values for polymer formulations.[a]

Polymer formulation	*S. aureus*		*E. coli*	
	MIC (wt%)	MBC (wt%)	MIC (wt%)	MBC (wt%)
Anionic MSs	> 0.7	> 0.7	> 0.7	> 0.7
Cationic PDADMAC	0.0005 ± 0.00003	0.001 ± 0.00006	0.0007[26]	0.0014[26]
Anionic complex (Z = 0.1)	0.0016 ± 0.00007	0.0022 ± 0.0001	0.003 ± 0.00017	> 0.003
Anionic complex (Z = 0.2)	0.00045 ± 0.00003	0.00084 ± 0.00004	0.0011 ± 0.00006	> 0.003
Cationic complex (Z = 1.2)	0.00024 ± 0.00002	0.00036 ± 0.00002	0.00024 ± 0.000016	0.00048 ± 0.00003

[a] The MIC and MBC values for the PDADMAC–MS complexes are shown as a weight percentage of PDADMAC.

thus showing the preservation of complex particles over the entire range of salt concentrations, including 0.1 mol dm^{-3} salt concentration for the antimicrobial testing. PDADMAC–MS complexes thereby interacted with microorganisms as a whole without pre-dissociation into the original components.

Table 1 contains the MIC and MBC values for the initial components, MSs and PDADMAC, and three PDADMAC–MS complexes: two anionic with $Z = 0.1$ and $Z = 0.2$ and cationic with $Z = 1.2$, as a weight percentage of the polymer reagent (wt%). The complexes of the specified compositions were taken since they remained stable (did not dissociate into the original components) in water–salt solutions (see above).

Anionic microspheres showed low toxicity to the both test microorganisms: the MIC and MBC values exceeded 0.7 wt% concentration, which was the maximum in our experiments. On the contrary, PDADMAC was highly toxic with the MIC value in the 0.0005–0.0007 wt% range and the MBC value in the 0.001–0.0014 wt% range, which was in agreement with the published data.[26] Thus, in terms of MIC/MBC values the cationic PDADMAC was by three orders of magnitude more toxic than the anionic MSs; therefore, a polymer concentration three orders of magnitude lower was required to produce a toxic effect.

The adsorption of PDADMAC on the MS surface could have different effects on the antimicrobial activity of the polycation: it could remain unchanged or increase, for example, due to the concentration of the polymer onto the MS surface, or, on the contrary, decrease or even be completely leveled due to the predominant role of non-toxic anionic groups. Therefore, one can see that the antimicrobial activity could depend on the amount of adsorbed polycation and the fraction of the MS surface area covered by polymer.

Look at the MIC and MBC data for PDADMAC–MS complexes presented in Table 1. Considering the extremely low toxicity of MSs, it is natural to believe that PDADMAC makes a decisive contribution to the overall toxicity of the complexes. Therefore, the MIC and MBC values for the complexes are shown as a weight percentage of PDADMAC.

The cationic complex with $Z = 1.2$ showed that the MIC and MBC values are 2–3 times lower than those for the individual polycation. This could result from the above-mentioned concentration of PDADMAC on the MS surface, which ensured greater penetration of the toxic polycation.

The distribution of the same amount of PDADMAC over more MSs led to the formation of anionic complexes with $Z = 0.1$ and 0.2 where only part of the MS surface was covered by polycation. Such a PDADMAC redistribution had a negligible effect on the MIC and MBC values of the resulting complexes which remained 2–3 orders of magnitude lower than the corresponding values for the anionic MSs.

Thus, the level of the PDADMAC–MS complex toxicity is determined by the total amount of polycation in the system and does not depend on the distribution of the polycation between MSs and its coverage of the MS surface (Figure 3). Both individual PDADMAC and PDADMAC–MS complexes show high toxicity towards gram-positive *S. aureus* and gram-negative *E. coli*. The results indicate the decisive role of polymeric toxins (PDADMAC) in the overall biological effect of their complexes with microplastics.

In summary, the individual PDADMAC and MSs show different behavior towards microorganisms: the former demonstrates a high antimicrobial activity to gram-positive *S. aureus* and gram-negative *E. coli*, and the latter are practically inert. Adsorption of PDADMAC on the MS surface leads to the formation of PDADMAC–MS complexes whose antimicrobial activity is determined by the total amount of polycation in the system and does not depend on the distribution of the polycation between the MSs. The antimicrobial activity of complexes is high and comparable with the activity of individual PDADMAC at the same concentration. It has been shown recently that polycationic toxins are able to migrate between negative polymer particles.[20] In relation to microplastics, the above results mean that the redistribution of adsorbed polycations between the particles has almost no effect on the toxicity of complex particles, which retain a significant negative impact on microorganisms.

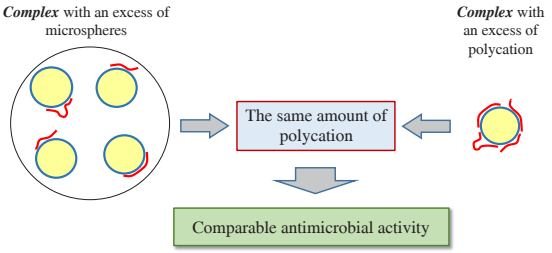


Figure 3 Schematical representation of the structure of PDADMAC–MS complex and its antimicrobial activity.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7619.

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