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Hybrid polycomplexes of anionic alginate with a synthetic cationic polymer: attractive and poisonous for microorganisms

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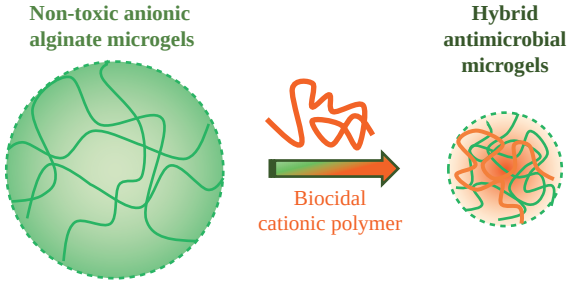
Negatively charged polycomplexes have been prepared by electrostatic complexation of synthetic cationic poly(diallyldimethylammonium chloride) with excess anionic sodium alginate. These polycomplexes exhibit pronounced antimicrobial activity due to the biodegradation of the alginate matrix and the release of the biocidal polycation. The results obtained are promising for the development of simultaneously attractive and toxic polymer constructs capable of destroying microorganisms.



Keywords: alginate, microgels, poly(diallyldimethylammonium chloride), negatively charged polycomplex, antimicrobial activity.

Polycomplexes formed by polyanion–polycation pairs have found application in biomedicine, pharmacology, cosmetology, food industry, *etc*.[1–5] Polycomplexes of natural compounds are of particular interest due to their low toxicity, high biocompatibility and biodegradability.[2–4] Alginate (ALG), an anionic copolymer of β-D-mannuronic and α-L-guluronic acids, is a very common natural polysaccharide.[6] This copolymer is used in drug delivery systems, tissue engineering and reparative medicine, cell immobilization and surface modification for biomedical applications, food industry, *etc*.[6–9] Recently, experimental data have appeared indicating the antiviral activity of ALG.[10,11] For tissue regeneration and tissue engineering, ALG hydrogels are predominantly used, the structure of which is similar to that of the extracellular tissue matrix.[7]

ALG is food for many microorganisms, including pathogens.[6,11–13] This fact can be used to develop biocidal formulations that are both nutritious and toxic to microorganisms. ALG in such formulations will act as an 'edible' matrix in which the toxic substance is distributed ('hidden'). After digestion of the polysaccharide matrix, the encapsulated biocide will begin to destroy surrounding microorganisms, especially the closest ones. It can be expected that microorganisms will prefer to interact with edible bioactive particles rather than synthetic ones. In this work, we tested this idea by modifying ALG microgels with the synthetic cationic polymer poly(diallyldimethylammonium chloride) (PDADMAC), characterized by pronounced biocidal properties.[14]

The polycomplexes were obtained† by adding a PDADMAC solution to a suspension of ionically cross-linked ALG microgels, which led to neutralization of the charge of ALG microgels, which was registered by measuring their electrophoretic mobility (EPM), a parameter associated with the surface charge of microgels, using microelectrophoresis (Figure 1, curve *1*). Complete neutralization of the ALG charge (EPM = 0) was observed at a molar ratio of cationic PDADMAC and anionic ALG units Z = [PDADMAC]/[ALG] = 0.9. Taking into account 5 mol% of Ca^{2+} ions in the original ALG, which electrostatically bound 10 mol% of carboxyl groups of ALG, the value of Z = 0.9 at EPM = 0 means that all free (not bound to Ca^{2+} ions) carboxyl groups of ALG microgels were electrostatically bound to cationic groups of PDADMAC. A further increase in the concentration of PDADMAC led to the formation of positively charged particles of the polycomplex due to the adsorption of a large amount of polycation, exceeding that required for neutralization.

In parallel, the particle size of the polycomplex was controlled by dynamic light scattering (Figure 1, curve *2*). Increasing the concentration of PDADMAC initially led to an increase in the size of the polycomplex due to neutralization of the charge of the

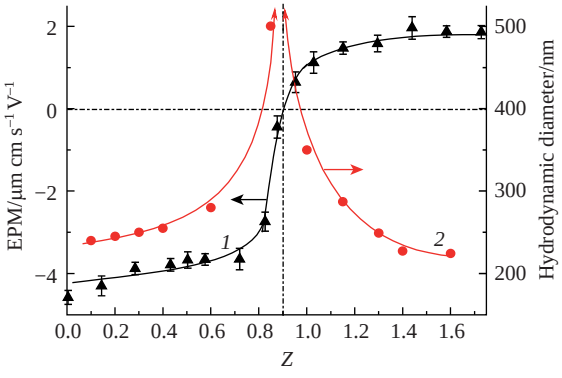


Figure 1 Dependences of (*1*) EPM and (*2*) hydrodynamic diameter of polycomplex particles on the Z ratio at [ALG] = 5×10^{-4} M in 0.01 M Tris buffer (pH 7).

† *Preparation of polycomplexes*. Sodium ALG containing 5 mol% Ca^{2+} ions (ISP, UK)[15] was dissolved in 0.01 M Tris buffer (pH 7) to give a suspension of ionically cross-linked ALG microgels. Then PDADMAC (M_w 470 kDa, CPS, USA) dissolved in Tris buffer (pH 7) was added to the ALG suspension.

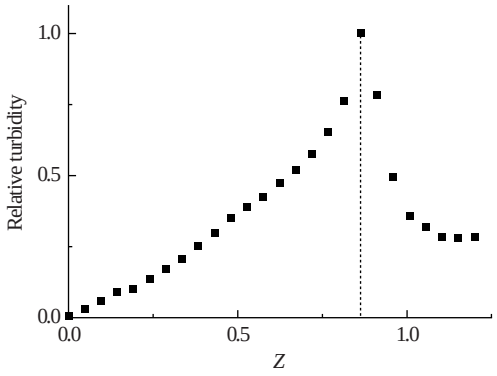


Figure 2 Dependence of the relative turbidity of a solution of the polycomplex in 0.01 M Tris buffer (pH 7) on the *Z* ratio at 500 nm and [ALG] = 10^{-3} M.

polycomplex particles and their aggregation. The size reached its maximum value at EPM = 0, and as the excess of PDADMAC increased, the particles decreased in size due to the abundant positive charge introduced by the adsorbed polycation.

The formation of polycomplex particles was reflected in the turbidity of the polycomplex solution (Figure 2). In general, the plot of turbidity *vs.* *Z* is similar to the plot of particle size *vs.* *Z* in Figure 1 (curve *2*) with a progressive increase in turbidity in the first (left) segment, reaching a maximum at $Z = 0.9$ and a sharp decrease in the second (right) segment. In the range $Z \leqslant 0.3$, the turbidity did not exceed 20%, which corresponded to particles with a size of 230–250 nm. Such particles carried a negative charge with an EPM of no lower than –4 μm cm s^{-1} V^{-1}, which ensured the resistance of the particles to aggregation.

Solutions of polycomplexes with $Z \leqslant 0.3$ were subjected to antimicrobial tests. The test cultures used were non-pathogenic strains of Gram-positive bacteria *Staphylococcus aureus* 209P and *Micrococcus luteus* NCIMB 13267, Gram-negative bacteria *Pseudomonas aeruginosa* 4.8.1 and *Escherichia coli* MG 1655 K12, as well as yeast *Yarrowia lipolytica* 367-2 (all samples from the collection of microorganisms of the FRC 'Fundamentals of Biotechnology' RAS).

The antimicrobial properties of aqueous solutions of polycomplexes were quantitatively characterized by the values of their minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against five test microorganisms according to previously described methods.[16,17] The MIC expresses the lowest concentration of the polycomplex that inhibits the growth of microorganisms in solution, and the MBC corresponds to the lowest concentration of the polycomplex that kills microorganisms. The MICs and MBCs for the three ALG–PDADMAC polycomplexes with *Z* of 0.1, 0.2 and 0.3, as well as the parent ALG and PDADMAC, are shown in Table 1 as the weight percentage (wt%) of the polymer reagent. As expected, individual ALG showed extremely low toxicity towards all five test microorganisms: MIC and MBC exceed the 1 wt% concentration, which was the maximum in our experiments (entries 13 and 14). In contrast, PDADMAC was found to be highly toxic with an MIC of 0.0002 to 0.001 wt% and an MBC of 0.0003 to 0.002 wt% (entries 15 and 16). These results were consistent with the published data.[7,18–20]

The MIC and MBC values for the polycomplexes (entries 1, 3, 5, 7, 9 and 11) are between the corresponding values for ALG and PDADMAC. The Gram-positive bacteria and yeast are the most sensitive to polycomplex formulations. The MIC and MBC values for polycomplexes reduce with increasing the PDADMAC content, gradually approaching the values for individual PDADMAC.

The different sensitivity of test microorganisms to the PDADMAC-based formulations is due to the structure of the cell wall. Gram-negative bacteria with a second (outer) membrane are most protected from biocides and are less sensitive to them. In contrast, Gram-positive bacteria and yeast have loose cell walls and are more vulnerable to hazardous chemicals.

Since ALG itself has been found to be nontoxic to all microorganisms, the toxicity of the polycomplexes can reasonably be attributed to the toxicity of the cationic component PDADMAC. For each polycomplex sample, the PDADMAC content was calculated, which allowed us to establish a correlation between the MIC and MBC values and the weight percentage of PDADMAC in the polycomplexes (entries 2, 4, 6, 8, 10 and 12). The MIC and MBC values thus obtained coincide or are close to the corresponding values for PDADMAC alone (entries 15 and 16). This experimentally confirms the decisive contribution of the polycation to the toxicity of polycomplexes.

Such behavior of the negatively charged 'semi-synthetic' ALG–PDADMAC polycomplexes differs significantly from the behavior of the negatively charged 'fully synthetic' polycomplexes formed by PDADMAC and anionic synthetic polymers/microspheres. The latter demonstrate no or negligible antimicrobial activity.[21,22] The high negative charge of fully synthetic polycomplexes and

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

Entry	Formulation	Parameter (wt%)	*P. aeruginosa*	*E. coli*	*S. aureus*	*M. luteus*	*Y. lipolytica*
1	[PDADMAC]/[ALG] ($Z = 0.1$)	MIC[b]	0.0230	0.0090	0.0060	0.0025	0.0060
2		MIC[c]	0.0020	0.0007	0.0005	0.0002	0.0005
3		MBC[b]	n/a	0.0350	0.0120	0.0034	0.0300
4		MBC[c]	n/a	0.0030	0.0010	0.0003	0.0030
5	[PDADMAC]/[ALG] ($Z = 0.2$)	MIC[b]	0.0120	0.0050	0.0035	0.0015	0.0035
6		MIC[c]	0.0020	0.0007	0.0005	0.0002	0.0005
7		MBC[b]	n/a	0.0200	0.0070	0.0020	0.0180
8		MBC[c]	n/a	0.0030	0.0010	0.0003	0.0030
9	[PDADMAC]/[ALG] ($Z = 0.3$)	MIC[b]	0.0090	0.0035	0.0025	0.0010	0.0025
10		MIC[c]	0.0020	0.0007	0.0005	0.0002	0.0005
11		MBC[b]	n/a	0.0130	0.0050	0.0014	0.0130
12		MBC[c]	n/a	0.0030	0.0010	0.0003	0.0030
13	ALG	MIC	> 1	> 1	> 1	> 1	> 1
14		MBC	> 1	> 1	> 1	> 1	> 1
15	PDADMAC	MIC	0.0010	0.0007	0.0005	0.0002	0.0005
16		MBC	0.0020	0.0014	0.0010	0.0003	0.0015

[a] All experiments were performed in triplicate. Statistical processing of experimental data was carried out using the Excel program with an estimation of the arithmetic mean and standard deviation. The significance of differences between the variants was considered by Student's *t*-test for $p < 0.05$. [b] The value refers to the polycomplex as a whole. [c] The value refers to the amount of PDADMAC in the polycomplex.

their resistance to biodegradation make such constructs insensitive to the action of microorganisms. Bacteria and yeast do not interact with such polycomplexes and are therefore not affected by the embedded polycationic biocide.

The situation changes upon passing to positively charged fully synthetic polycomplexes. In this case, the positive charge makes possible the electrostatic interaction of the polycomplexes with cells, and the polycation exposed on the outer surface of the polycomplex particles has a biocidal effect on the cells.[21,22]

The semi-synthetic ALG–PDADMAC polycomplexes exhibit antimicrobial activity even with a negative charge. It follows that microorganisms destroy the ALG matrix and fall under the action of the hidden polycation. It's reminiscent of the Trojan horse of ancient Greek legends: a secret plan disguised as a gift.[23] In both cases, an attractive outer shell hides the dangerous filling.

In summary, negatively charged polycomplexes were prepared by electrostatic complexation of synthetic cationic PDADMAC with an excess of native anionic ALG. The semi-synthetic polycomplexes exhibit pronounced antimicrobial activity due to the biodegradation of the ALG matrix and the release of the biocidal polycation. The approach is promising for the preparation of simultaneously attractive and poisonous polymer constructs capable of destroying microorganisms. It is expected that application of the formulations to the surface and subsequent drying will lead to the formation of coatings with a prolonged biocidal effect. PDADMAC itself forms fragile films, and its complexation with ALG will improve the mechanical properties of the coatings while maintaining their biocidal properties.

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