

**Molecular weight of polyanion affects the biological activity of interpolycomplexes**

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**A.** Poly(diallyldimethylammonium chloride) (PDADMAC) with an average molecular mass  $M_w = 470$  kDa (CPS Chem. Com. Inc.) was used as a cationic polymer; two sodium polyacrylate samples with  $M_w = 8$  (PANa1) and 250 kDa (PANa2) (Sigma-Aldrich) were used as anionic polymers. PDADMAC was dissolved in  $10^{-3}$  M Tris buffer solution with pH 7 additionally containing  $10^{-2}$  M NaCl and mixed with a sodium polyacrylate (PANa) solution in the same buffer. The mixing resulted in an electrostatic PDADMAC-to-PANa complexation and formation of IPECs, stabilized by ionic bonds between oppositely charged groups of PDADMAC and PANa.<sup>S1,S2</sup> The salt solution provided an equilibrium distribution of PANa chains between PDADMAC chains as described earlier.<sup>S3</sup> Concentrations of polymers were expressed in moles of quaternary amino PDADMAC groups  $[N^+]$  and carboxylic PANa groups  $[COO^-]$  per liter.

**B.** The hydrodynamic diameter of the particles was measured using dynamic light scattering at a fixed scattering angle ( $90^\circ$ ) in a thermostatic cell ( $22^\circ\text{C}$ ) with a ZetaPlus instrument (Brookhaven, USA), their electrophoretic mobility (EPM) using laser microelectrophoresis with a ZetaPlus instrument (Brookhaven, USA). The results were processed using software provided by the manufacturer. All experiments were performed with 5-7 repetitions. Statistical data processing was carried out with the Excel program.

**C.** Anionic palmitoyloleoylphosphatidylserine ( $\text{POPS}^{1-}$ ) and zwitterionic dioleoylphosphatidylcholine (DOPC) (Avanti, USA, Houston, TX) were used as received. Liposomes were prepared by sonication with a standard protocol.<sup>S4</sup> The required amount of lipids was dissolved in chloroform; the organic solvent was removed under vacuum with Laborota-4000 (Heidolph, Germany)) at  $30^\circ\text{C}$ . The lipid film was dispersed by vortex in a buffer solution. The resulting dispersion was treated with ultrasound using an ultrasonic homogenizer (Drawell JY92-

IIN, China). The resulting liposomes were purified from titanium dust by centrifugation for 5 minutes at 11,000 rpm. Molar ratio of lipids [POPS<sup>1-</sup>]/[DOPC] in liposomes was 20/80. The average liposome diameter was of 85±5 nm, electrophoretic mobility was of -3.75± 0.1 (µm/s)/(V/cm).

**D.** Minimum inhibitory concentrations (MIC) for polymer formulations were determined towards gram-negative bacteria *Pseudomonas aeruginosa* 4.8.1 using the standard procedure.<sup>S5</sup> Briefly, an aqueous polymer solution was added to glass test-tubes with M9 medium – an aqueous solution, which contained a mixture of glucose and inorganic salts with a total salt concentration of about 0.08 M; the polymer concentration ranged from 0 to 2 wt%. Then, the tubes were inoculated with the bacteria *P. aeruginosa* and placed on a shaker at 28 °C; 2 days after, the growth of microorganisms was assessed. The lowest polymer concentration, at which no growth of the test cultures was observed visually, was taken as MIC.

## References

- S1 O. A. Novoskoltseva, E. V. Chernikova, V. B. Rogacheva and A. B. Zezin, *Polymer Science Series B*, 2015, **57**, 132.
- S2 I. G. Panova, A. Yu. Lokova, D. V. Bagrov, N. G. Loiko, Yu. A. Nikolaev and A. A. Yaroslavov, *Mendeleev Commun.*, 2023, **33**, 562.
- S3 V. A. Kabanov, *Russ. Chem. Rev.*, 2005, **74**, 3.
- S4 V. V. Spiridonov, I. G. Panova, A. V. Sybachin, V. V. Kuznetsov, M. I. Afanasov, Y. A. Alekhina and A. A. Yaroslavov, *Polymer Science Series A*, 2019, **61**, 296.
- S5 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.