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Mendeleev Commun., 2022, **32**, 561–563

Mendeleev Communications

Hyperbranched Kaustamin as an antibacterial for surface treatment

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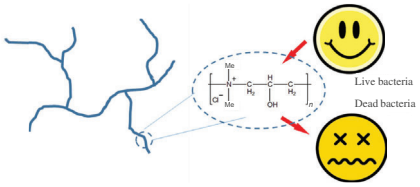
DOI: 10.1016/j.mencom.2022.07.042

Hyperbranched Kaustamin as a commercially available cationic flocculant adsorbs on a glass surface with formation of the polymer film. Kaustamin has antimicrobial effect on Gram-negative and Gram-positive bacteria, its film on glass keeps integrity and shape after absorption of water from air, which make the polymer appropriate as an antibacterial cover.



Keywords: hyperbranched Kaustamin, polycation, adsorption, coating, antimicrobial activity, atomic force microscopy.

Since the COVID-19 pandemic and subsequent general weakening of the immune system, the demand for antimicrobial coatings to avoid bacterial spread is of particular importance.[1] Many indoor surfaces that require antimicrobial processing contain silanol groups, *e.g.*, window and furniture glass as well as ceramic floor.[2,3] Antimicrobials used to treat them include polymer-based biocides, which are applied from an aqueous phase and demonstrate high adhesion to the processed surface.[1,4] An example is polycationic polymers,[5,6] which interact with a negatively charged membrane of bacterial cell and induce structural rearrangements in the lipid bilayer leading to disruption of the vital activity of bacteria or their death.[7,8] The polycationic biocides are typically obtained by complicated synthesis or have a high commercial price. That is why cheap polymers of this type are of particular interest for market.[9]

Non-expensive mass market polycations are typically used as flocculants for water treatment as well as components of soil structurants and drilling fluids,[5,10,11] an example is Kaustamin, which represents a product of epichlorohydrin condensation with dimethylamine. To attain high molecular weight and branched structure, ethylenediamine is also added (Figure S1, see Online Supplementary Materials).[12] As a result, the monomer unit of hyperbranched Kaustamin (HKAU) contains quaternary amino and hydroxyl groups, which allow its further modification with low molecular weight biocide moieties to increase the effect. In this work, antimicrobial activity of HKAU as well as its ability to form a coating on glass surface were explored.

HKAU (FL2850, lot LCAV 11/6660, SNF Vostok, Russia) was used as an initial 50 wt% solution. Its molecular weight was determined by static light scattering in 0.15 M aqueous NaCl. The scattering intensities for the solutions with polymer concentrations of 0.06–0.14 mg ml^{-1} at angles θ of 30–120° were recorded. The corresponding Zimm plot is shown in Figure 1, where KC is material constant at concentration C, R_θ is the excess Rayleigh ratio between the polymer and the pure solvent at an angle θ, while A represents a virial coefficient. Extrapolation of the data to zero angle and zero concentration gives the inverse value of weight average molecular mass M_w and radius of gyration R_g. The M_w and R_g values were estimated to be 8.55×10^5 Da and 62 nm, respectively, while hydrodynamic radius R_h was calculated as 160 nm using the diffusion coefficient from dynamic light scattering.[13]

Then the antibacterial activity of HKAU was investigated by the double fluorescent protein reporter procedure described by our group.[14] Solution of HKAU in distilled water (2.5 μl, 20 mg ml^{-1}) was applied to an agar plate with Lysogeny broth (LB) covered with a lawn of Gram-positive *Bacillus subtilis* or Gram-negative *Escherichia coli* Δ*tolC* strain. Plates were incubated at 37 °C for 18 h and then scanned by ChemiDoc (Bio-Rad) in the Cy3 and Cy5 blot modes with emission filters at 605 ± 50 and 695 ± 50 nm, respectively.

The strain of *E. coli* lacking the *tolC* gene represents a good tool to determine the mechanism of HKAU action, since as it has been demonstrated by our group, in the presence of a compound that causes cellular SOS response or leads to violation of protein biosynthesis, the accumulation of two types of proteins occurs, namely red fluorescent protein (RFP) and/or the Katushka2S one, with their genes being placed under the SOS response promoter and a modified tryptophan attenuator, respectively.[14] The results with *E. coli* are shown in Figure 2, where HKAU seems to act like erythromycin, *i.e.*, it presumably affects the

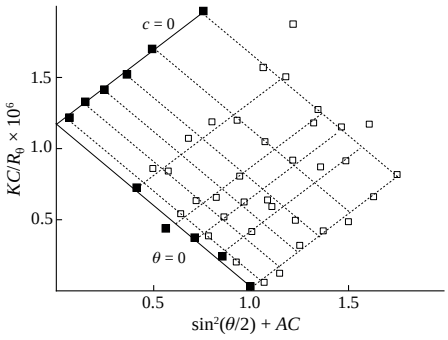


Figure 1 Zimm plot for HKAU light scattering in 0.15 M solution of NaCl.

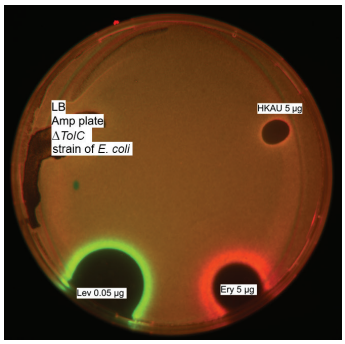


Figure 2 Screening for bacterial growth inhibition. HKAU together with erythromycin (Ery) and levofloxacin (Lev) as positive controls were applied to the agar plate surface covered with *E. coli* Δ*tolC* strain.

translation stage. In Figure 2, the area of inhibition zone for HKAU is smaller than for the erythromycin and levofloxacin positive controls, the reason may be poor diffusion of the hyperbranched polymer into the solid support compared with a liquid medium. Therefore, minimal inhibition concentration (MIC) was estimated (for details, see Online Supplementary Materials)[15] using Gram-negative *E. coli* K-12 and Δ*tolC* strains as well as Gram-positive *B. subtilis* in the LB and Tryptic Soy Broth (TBS) media (Table 1). The latter medium is favorable for biofilm formation.[6,16]

HKAU demonstrates higher antimicrobial activity for Gram-positive *B. subtilis* compared with Gram-negative *E. coli*, though the activity is lower than the one for the conventional anitibacterial drug. However, it is the ability of the polymer-based antibiotics to form continuous coatings on the surfaces that makes them promising for long term antimicrobial protection.

The formation of HKAU cover on a glass surface was explored after deposition of its solution (200 μl, 20 mg ml^{-1}) to a 2.89 cm^2 freshly cleaned and weighed glass coverslip with subsequent drying in air. The weight of the polymer film was determined from the weight difference. Then 200 μl of water was applied to the glass so that it completely covered the surface of the film. After incubation for 1 min the liquid was eliminated, the sample was left to dry completely and weighed again. Thus, the resistance of the film towards wash-off was estimated from the weight loss. As an average of three experiments, ~80% HKAU was removed from the glass surface after the 1st cycle of wash-off, while almost complete loss was detected after the 3rd cycle (Figure S3). Thus, deposition of concentrated solution of the polymer on glass surface results in a film, which is very sensitive to the impact of water. So, further an ability of the HKAU film to absorb water from environment was tested. The film was prepared in a Petri dish, dried in air with 5% relative humidity and weighed, this value was used as a reference. The weight gain of the film after incubation in the environment with controlled humidity was used to estimate the capacity of the film to absorb water from air (for details, see Online Supplementary Materials). Increase in humidity from 15 to 62% results in progressive elevation of the film weight from 5 to 30%. The film adsorbed on the glass surface did not change its shape during the experiment. Therefore, absorbance of water even at high relative humidity

Table 1 MIC values for HKAU and erythromycin.

Bacterial strain	Medium	MIC for HKAU/ μg ml^{-1}	MIC for erythromycin/ μg ml^{-1}
B. subtilis	LB	12	1
B. subtilis	TSB	< 60	1
E. coli K12	LB	500	125
E. coli Δ*tolC*	LB	200	4

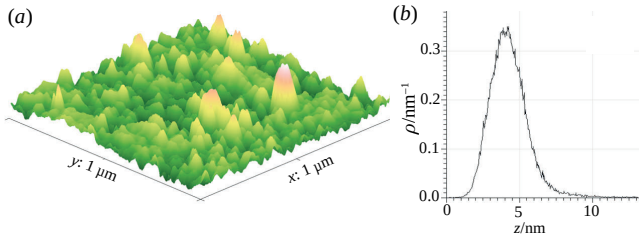


Figure 3 (*a*) AFM image of the HKAU layer and (*b*) size distribution of the HKAU molecules on a glass surface.

can not be considered as a factor resulting in elimination of the HKAU macromolecules from the film.

However, it is important to estimate the presence of adsorbed macromolecules on the glass surface after several wash-off cycles, which is below the threshold of the weight measurements using a balance. Thus, a freshly cleaned 2.89 cm^2 glass coverslip was dipped into an aqueous solution of HKAU with a concentration of 20 mg ml^{-1} and kept there for 2 min to ensure exhaustive adsorption of the polymer. Then the sample was placed in a beaker with distilled water, shaken vigorously for 2 min to eliminate the HKAU macromolecules from the surface, dried in air and then analyzed using atomic force microscopy (AFM). The relief recorded is attributed to a monolayer of HKAU [Figure 3(*a*)]. The dense relief has an average height of 4.2 nm {[Figure 3(*b*)], for details, see Online Supplementary Materials}, which corresponds to formation of loops and tails by the HKAU macromolecules. So, we emphasize that the layer of HKAU remains on the glass surface even after intensive wash-off.

In summary, commercially available polycation flocculant HKAU was demonstrated to adsorb on a glass surface with formation of the polymer film, which could absorb up to 30% water related to its dry weight from an environment having relative humidity up to 62%. This absorption did not change the integrity and shape of the HKAU film. Intensive wash-off led to consequent removal of HKAU from the glass surface, however its monolayer retained forming dense relief with an average height of 4.2 nm. With the fact that HKAU reveals antibacterial effect, it is suggested to use the polymer for antimicrobial treatment of the glass and glass-like surfaces such as elements of furniture or floor/wall tiles.

This work was supported by the Ministry of Science and Higher Education of the Russian Federation (project no. 075-15-2020-775) and partially supported by the Russian Science Foundation (grant no. 21-64-00006 to V. I. M. and I. A. O. for antibacterial activity measurements).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.07.042.

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Received: 7th February 2022; Com. 22/6800