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High antibacterial activity and low toxicity of pyridoxal derivatives of chitosan and their nanoparticles

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In this study, we used crab shell chitosan (Bioprogress, Russia) with a viscosity-average molecular weight (M_W) of 3.7×10^4 and a degree of acetylation of 25 %, viscosity-average molecular weight (M_W) of 6.9×10^4 and a degree of acetylation of 25 % and viscosity-average molecular weight (M_W) of 17.8×10^4 and a degree of acetylation of 25 % (determined from ^{1}H NMR), pyridoxal, $NaBH_4$ (Aldrich). Other chemicals, solvents and materials were obtained from commercial sources and were used as received.

The viscosity of the chitosan solutions in 0.3 M of NaCl/2% acetic acid was measured at 20 °C in viscometer. The intrinsic viscosity of the samples was calculated by extrapolation of the dependence $\ln(\eta_r)\times C^{-1}$ to an infinite dilution using the least squares method [1].

The viscosity-average molecular weight of chitosan sample was calculated using the Mark–Kuhn–Houwink–Sakurada equation: $[\eta] = K\times M_W^{\alpha}$, where $[\eta]$ is the intrinsic viscosity and K and α are empirical constants ($K = 1.34\times10^{-3}$, $\alpha = 1.02$) [1].

The ^{1}H NMR spectra were recorded on a Bruker Avance II spectrometer (Germany) at operating frequencies of 400 MHz and 100 MHz, respectively.

The apparent hydrodynamic diameter and ζ-potential of nanoparticles in water were estimated at room temperature (about 20 °C) using a Photocor Compact-Z instrument (Russia) at λ=659 nm and θ=90°.

SEM images were obtained by electron microscope JEOL JSM - 6490LV at 15kV, SEM detector, electron beam size 30, in high vacuum. The test samples were coated with 20nm (40sec at 40mA) with a platinum layer in a JEOL auto fine coater JFC - 1600.

Antibacterial activities were investigated as described elsewhere [2].

Toxicity was studied as described elsewhere [3].

Preparation of pyridoxal chitosan derivatives

Chitosan (0.1 g) was dissolved in 0.1 M HCl (10 ml), then pyridoxal (1, 1.6 or 5 equiv.) was added and the reaction mixture was stirred at 25 °C for 3 hours. After this, $NaBH_4$ (2, 3.2 or 10 equiv.) was added and the reaction was stirred at 25 °C for 5 hours. The resulting polymers were precipitated by acetone (30 ml), dissolved in distilled water, and, finally, freeze-dried.

Preparation of nanoparticles

Nanoparticles were prepared by dissolving 20 mg of pyridoxal chitosan derivative in 20 mL of distilled water. After 3 h of stirring, 0.25% aqueous sodium tripolyphosphate (TPP) solution was rapidly added volume, see Table 2 in the main text). The resulting nanoparticle suspension was centrifuged at 17000 rpm and 2 °C for 40 min using an Optima MAX-XP centrifuge (Beckman Coulter). The pelleted nanoparticles were redispersed in 20 mL of water and lyophilized.

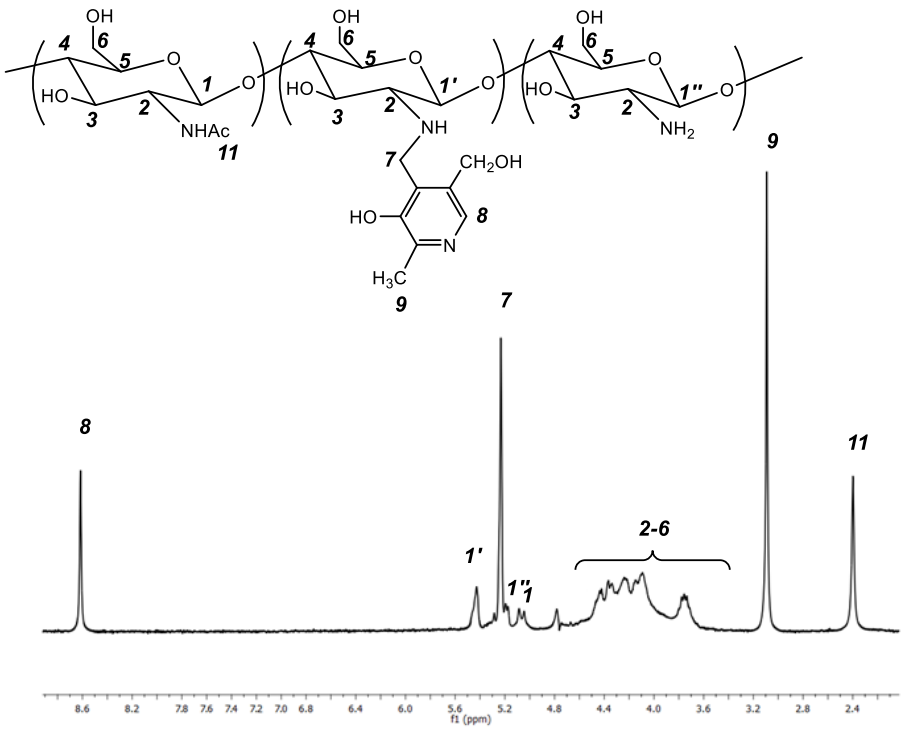

Figure S1 ^{1}H NMR spectrum of the prepared chitosan derivative **P-CS-III-M**.

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