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

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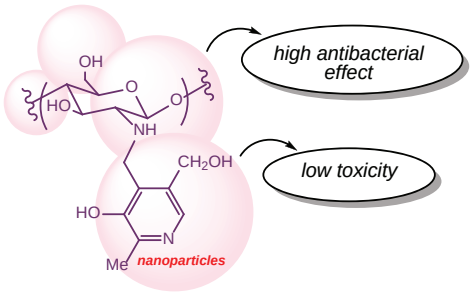
The pyridoxal derivatives of chitosan with various degrees of substitution (DS) were synthesized from low-, moderate- and high-molecular-weight chitosans by their reaction with pyridoxal followed by treatment with $NaBH_4$. The derivative of moderate molecular weight and high DS demonstrated a maximum antibacterial activity against *S. aureus* and *E. coli*. The nanoparticles of this derivative obtained by ionic gelation are nontoxic, and they exhibit a high *in vitro* antibacterial effect, which slightly exceeds that of ampicillin and gentamicin.

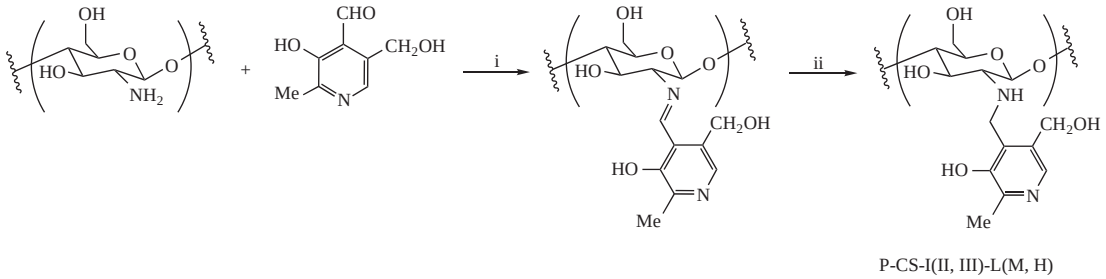


Keywords: chitosan, pyridoxal, nanoparticles, antibacterial activity, low toxicity.

Chitosan is a biocompatible, biodegradable and nontoxic natural polymer, which consists of *N*-acetyl-D-glucosamine and D-glucosamine units.[1] A wide range of chitosan-based multifunctional materials were developed,[2–7] including low-toxicity antibacterial systems, as compared with conventional synthetic antibiotics.[8–10] However, the antibacterial activity of chitosan is limited by its moderate cationic density, which can be increased by introduction of substituents into the chitosan macromolecule.

In this study, we prepared pyridoxal derivatives of chitosan (Scheme 1) since pyridoxal is a nontoxic compound and the introduction of a pyridoxal moiety into the chitosan backbone increases the cationic density of chitosan. The resulting polymers were characterized by ^{1}H NMR spectroscopy (Figure 1). The degree of substitution (DS) of the polymers was calculated as DS = I(1′), while I(12) = 0.75 (I is the integral intensity of corresponding protons in the ^{1}H NMR spectrum; for proton numbering, see Online Supplementary Materials).

Thus, starting from low, medium and high molecular weight chitosans (3.7×10^4, 6.9×10^4 and 17.8×10^4 Da, respectively; the degree of acetylation, 25%), we prepared pyridoxal derivatives with low, medium and high degrees of substitution (about 15, 40 and 60%, respectively) (Table 1**)**. In the sample names, P refers to pyridoxal; CS refers to chitosan; I, II and III refer to low, moderate and high degrees of substitution, respectively; and H, M and L refer to high, medium and low molecular weight derivatives of chitosan, respectively. For example, P-CS-III-L is a pyridoxal derivative of chitosan with a high degree of substitution and a low molecular weight.



Scheme 1 *Reagents and conditions*: i, HCl (0.1 M aq.), 25 °C; ii, $NaBH_4$, H_2O.

Table 1 Degree of substitution, antibacterial activity and toxicity of the prepared polymers and their nanoparticles.

Sample	Degree of substitution	*S. aureus*	*E. coli*	Cell viability (%) at sample concentration (μg ml^{-1}) [a]		
		Inhibition zone/mm [a]		10	300	1000
Chitosan	–	13.2 ± 0.1	10.7 ± 0.3	100	96	65
P-CS-I-L	0.15	15.0 ± 0.3	12.3 ± 0.1	99	93	63
P-CS-I-M	0.16	20.8 ± 0.2	18.8 ± 0.4	98	94	62
P-CS-I-H	0.16	15.1 ± 0.1	12.9 ± 0.1	99	94	63
P-CS-II-L	0.42	20.1 ± 0.3	13.6 ± 0.2	99	93	63
P-CS-II-M	0.41	22.8 ± 0.2	20.2 ± 0.4	98	93	62
P-CS-II-H	0.43	18.1 ± 0.3	14.3 ± 0.2	97	94	64
P-CS-III-L	0.62	23.7 ± 0.4	16.5 ± 0.2	98	93	63
P-CS-III-M	0.62	27.0 ± 0.1	20.9 ± 0.2	97	92	62
P-CS-III-H	0.63	24.2 ± 0.1	16.6 ± 0.1	98	93	62
Ampicillin		30.2 ± 0.2	–	60	43	31
Gentamicin	–	–	22.1 ± 0.2	58	37	20

[a] Mean value ± SD, n = 3.

We tested *in vitro* the antibacterial activity of the pyridoxal derivatives of chitosan against the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli* and compared it with that of the commercial antibiotics ampicillin and gentamicin using an agar diffusion method. The diffusion of an antibiotic into agar leads to the formation of a inhibition zone of the growth of microorganisms around the site of antibiotic application to the agar with fresh inoculation of bacteria. The most active antibiotic corresponds to the largest inhibition zone.

The antibacterial activity of the resulting polymers depends on both the molecular weight of the chitosan derivative and its degree of substitution. The medium molecular weight polymers were most effective. Moreover, an increase in the degree of substitution led to an increase in antibacterial activity against both *S. aureus* and *E. coli*. This fact can be explained by the following reasons: (*1*) an increase in the cationic density of the polymer, caused by both an increase in the fraction of secondary amino groups in the derivative macromolecule and a symbatic increase in the number of positively charged quaternized nitrogen atoms and (*2*) an increase in the fraction of hydrophobic moieties in the macromolecule responsible for hydrophobic interactions with the surface of bacterial cells.

Different polymer nanoparticles can exhibit enhanced antibacterial activity, as compared with that of corresponding polymers in their native forms.[11] Thus, nanoparticles are of increasing interest as an alternative to conventional antibacterial drugs. We prepared nanoparticles from the most effective antibacterial chitosan derivative P-CS-III-M using ionic gelation with sodium tripolyphosphate (Table 2, Figure 1). The nanoparticles exhibited a unimodal particle size distribution.

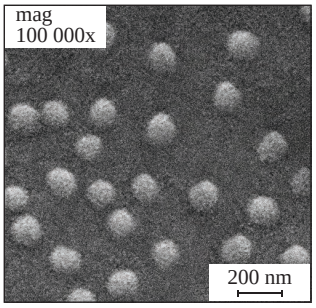


Figure 1 SEM image of NP-2 nanoparticles.

The antibacterial activity of the nanoparticles against both *S. aureus* and *E. coli* demonstrated strong dependence on the particle size and ζ potential. Table 2 shows that the most effective nanoparticles (see inhibition zones) had a moderate size (about 100 nm) and a high positive ζ potential (about +69 mV). The nanoparticles with the lowest ζ potential (about +32 mV) and a size of 300 nm had the lowest antibacterial effect. The nanoparticles with higher sizes (500 and 800 nm) and ζ potentials (+51 and +64 mV, respectively) possessed moderate antibacterial activity. Thus, the NP-2 nanoparticles were the most active antibacterial systems among those obtained in this work. Their antibacterial activity was comparable with or even higher than that of ampicillin and gentamicin (see Table 1).

An important advantage of chitosan over other antibacterial agents is its low toxicity, but this is not always true with all chitosan-based systems. Actually, each new chitosan derivative designed for biomedical applications should be characterized in terms of toxicity. In this study, we used a so-called MTT test, a colorimetric method for estimating the number of viable cells in culture, to assess the toxicity of TMAB-CS. The basis of this method is that NADPH-dependent dehydrogenases of living cells efficiently reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to form a purple formazan product. Thus, the color intensity is proportional to the viability of the cell culture. The highest cell viability corresponds to the lowest toxicity of the test sample. We compared the toxicity of the starting chitosan in a hydrochloride form with the toxicity of the antibacterial chitosan derivatives (see Table 1, cell viability) and their nanoparticles (see Table 2, cell viability) and found that the toxicity of the effective chitosan-based antibacterial systems was similar to that of the original chitosan. This fact is not surprising since the obtained derivatives are the addition products of natural nontoxic pyridoxal to the natural nontoxic polymer chitosan. Thus, we prepared an effective nontoxic chitosan-based antibacterial nanosystem NP-2, which is comparable with the antibiotics ampicillin and gentamicin.

Table 2 Characterization and antibacterial activity of nanoparticles.[a]

Sample	$2R_h$/nm	V_{TPP}/ml	PDI	ζ/mV	*S. aureus*	*E. coli*	Cell viability (%) at sample concentration (μg ml^{-1})		
					Inhibition zone/mm		10	300	1000
NP-1	63 ± 5	0.70	0.29 ± 0.03	72.1 ± 0.2	29.0 ± 0.1	20.9 ± 0.1	97	91	62
NP-2	105 ± 3	1.00	0.22 ± 0.03	69.2 ± 0.2	33.2 ± 0.2	23.0 ± 0.3	98	93	64
NP-3	201 ± 1	1.30	0.23 ± 0.04	55.1 ± 0.3	29.3 ± 0.4	21.4 ± 0.2	98	92	63
NP-4	306 ± 3	1.65	0.18 ± 0.05	32.0 ± 0.3	26.4 ± 0.2	18.9 ± 0.1	99	93	63
NP-5	509 ± 1	1.95	0.15 ± 0.02	51.5 ± 0.1	27.1 ± 0.1	19.6 ± 0.2	97	93	63
NP-6	811 ± 2	2.80	0.16 ± 0.03	64.2 ± 0.4	27.2 ± 0.2	19.8 ± 0.3	97	92	62

[a] Mean value ± SD, n = 3; $2R_h$ is the mean hydrodynamic diameter; V_{TPP} is the volume of a sodium tripolyphosphate solution; PDI is the polydispersity index; and ζ is the ζ potential.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2021.07.022.

References

1 G. Najafpour and M. Khorami, *Chem. Biochem. Eng. Q.*, 2012, **26**, 217.
2 E. Stoleru, S. B. Munteanu, R. P. Dumitriu, A. Coroaba, M. Drobotă, L. F. Zemljic, G. M. Pricope and C. Vasile, *Iran. Polym. J.*, 2016, **25**, 295.
3 W. Lan, S. Wang, Z. Zhang, X. Liang, X. Liu and J. Zhang, *Int. J. Biol. Macromol.*, 2021, **168**, 105.
4 Z. Dong, H. Cui, Y. Wang, C. Wang, Y. Li and C. Wang, *Carbohydr. Polym.*, 2020, **227**, 115338.
5 X. Chen, Y. Zhao, Y. Zhang, A. Lu, X. Li, L. Liu, G. Qin, Z. Fang, J. Zhang and Y. Liu, *Mater. Lett.*, 2019, **237**, 185.
6 A. Bhowmick, P. Jana, N. Pramanik, T. Mitra, S. L. Banerjee, A. Gnanamani, M. Das and P. P. Kundu, *Carbohydr. Polym.*, 2016, **151**, 879.
7 M. Alizadeh-Sani, M. Tavassoli, D. J. McClements and H. Hamishehkar, *Food Hydrocolloids*, 2021, **111**, 106237.
8 A. S. Kritchenkov, A. R. Egorov, A. P. Dysin, O. V. Volkova, L. A. Zabodalova, E. P. Suchkova, A. V. Kurliuk and T. V. Shakola, *Int. J. Biol. Macromol.*, 2019, **137**, 592.
9 A. S. Kritchenkov, A. R. Egorov, O. V. Volkova, I. S. Kritchenkov, A. V. Kurliuk, T. V. Shakola and V. N. Khrustalev, *Int. J. Biol. Macromol.*, 2019, **139**, 103.
10 A. S. Kritchenkov, A. I. Kurachenkov, A. R. Egorov, N. Z. Yagafarov, E. A. Fortalnova, N. N. Lobanov, A. P. Dysin, A. S. Khomik and V. N. Khrustalev, *Mendeleev Commun.*, 2020, **30**, 642.
11 M. Chandrasekaran, K. D. Kim and S. C. Chun, *Processes*, 2020, **8**, 1173.

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