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Molecular weight and pH aspects of the efficacy of oligochitosan against methicillin-resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

Oligochitosan samples varying in molecular weight (M_w) and having narrow polydispersities were prepared by means of depolymerization of chitosan in hydrochloric acid, and their antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) was measured at pH values 5.5–8.0. The antibacterial testing of oligochitosans obtained showed that oligochitosans having M_w in the range of 0.73–20.0 kDa could be used both at slightly acidic and neutral pH values, and that the activity against MRSA remained moderate for oligochitosan samples having M_w about 3–5 kDa even at slightly basic pH values. The self-assembling behavior of oligochitosan macromolecules in the dilute solution at various pH values as a function of chain length was investigated. At first it was shown that oligochitosans formed supramolecular aggregates in dilute solutions below the critical pH value 6.5. Despite the aggregation phenomenon, the formation of nano-sized aggregates did not prevent oligochitosan from demonstrating the bactiostatic activity.

1. Introduction

Among Gram-positive bacteria the mostly collected in hospitals, public buildings, and aircraft cabins is Gram-positive *Staphylococcus aureus* that can cause a wide variety of diseases in humans and animals. *S. aureus* is becoming more and more resistant to many commonly used antibiotics including penicillin, amoxicillin, tetracycline, erythromycin, linezolid, vancomycin, and methicillin (Gandara, 2006). Increased problems with human allergy also have been observed in the patients receiving antibiotic agents for treatment. As a result, benefits and safety of many biocides are the subjects of debates among regulators specializing in medicine, food, cosmetics, environmental sciences, and toxicology (Donadio, Maffioli, Monciardini, Sosio, & Jabes, 2010). Therefore, there is a need for new non-toxic biocides that could be active against broad spectrum of invasive and noninvasive human pathogens and could reduce the level of administration of classic antibiotics.

Chitosan produced by a partial or complete deacetylation of chitin represents a collective name for a group of polysaccharides consisting of glucosamine and *N*-acetylglucosamine or glucosamine only. Chitosan and chitooligosaccharides have attracted considerable interest due to their different biological activities (Xia, Liu, Zhang, & Chen, 2011). Numerous investigations of antimicrobial activity of chitosan, its derivatives and analogues named oligochitosan and chitooligosaccharides against many bacteria, including *S. aureus* (Muzzarelli et al., 1990), filamentous fungi and yeasts have been published so far, and nowadays it is commonly accepted that the activity depends on molecular weight (M_w), degree of deacetylation (DD), target microorganism, and experimental conditions. As to DD, the higher DD is, the higher activity occurs. On the other side, the controversial evidences for a correlation between biocidal activity and M_w of chitosan have been found so far. It was shown in some studies that the increase in chitosan molecular weight led to the decrease in biocidal activity of chitosan (Hernández-Lauzardo et al., 2008; Jung, Chung, & Lee, 2002; Tikhonov et al., 2006; Xu, Zhao, Han, & Du, 2007; Yun, Kim, & Lee, 1999; Zheng & Zhu, 2003). In the others an increased activity of high molecular weight chitosans in comparison with low molecular weight chitosans was found (Hirano & Nagao, 1989; Kim, Thomas, Lee, & Park, 2003; Li, Feng, Yang, Wang, & Su, 2008; Lin, Lin, & Chen, 2009; Liu, Guan, Yang, Li, & Yao, 2001; Qin et al., 2006; Shahidi, Arachchi, & Jeon, 1999; Zhang, Tan, Yuan, & Rui, 2003). It was only ones that the bell-like dependence of fungistatic activity versus molecular weight was found (Tikhonov et al., 2011). The M_w-activity relationship is also found dependent on the

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target microorganism: the lower molecular weight of chitosan is, the stronger antimicrobial effect against Gram-negative bacteria is. In the case of Gram-positive bacteria, the effect of higher molecular weight of chitosan is stronger than that of lower molecular weight chitosan (Eaton, Fernandes, Pereira, Pintado, & Malcata, 2008; Fernandes et al., 2008).

In our opinion, the controversial results concerning biocidal activity and its correlation with M_w of chitosan have been found mainly because so far most investigators either have used only few chitosan samples, or molecular weight distribution/polydispersity have not been taken into account. That is, some of the biological effects reported for chitosan may be caused by the presence of lower molecular weight chitosan macromolecules and chitooligosaccharides. This means that before investigating each sample also must be characterized by its polydispersity. The controversy may also be caused by presence of by-products and variation in the chemical structure of end-units and acetyl-group distribution along polysaccharide chains due to the differences in the methods used for depolymerization of HMW chitosan.

In this paper, we describe a preparation of well-characterized oligochitosan samples varying in M_w and having a narrow polydispersity as well as their bactericidal activities against methicillin-resistant (MRSA) *S. aureus* mainly focusing on the M_w–pH-activity relationship. Also, we describe our first results of the self-assembling behavior of oligochitosan macromolecules in the dilute solution at various pH values as a function of chain length.

2. Materials and methods

2.1. Preparation of oligochitosan

Low molecular weight (LMW) chitosan (M_w 70 kDa, DD 80 mol%) used for preparation of oligochitosan samples was purchased from ALDRICH. LMW chitosan was hydrolyzed in hydrochloric acid at 70 °C. Oligochitosan hydrochloride was precipitated with ethanol and dried in vacuum over sodium hydroxide. The yield of oligochitosan hydrochloride was in the range of 20–90% depending on M_w of the final product.

2.2. Molecular weight and polydispersity

The weight-average (M_w), number-average (M_n) molecular weight, and polydispersity indexes (PI = M_w/M_n) of LMW chitosan and oligochitosan samples were determined by HP SEC method described in Lopatin, Derbeneva, Kulikov, Varlamov, & Shpigun (2009).

2.3. Degree of deacetylation

Degree of deacetylation (DD, mol%) was determined by ^{1}H NMR method (Hirai, Odani, & Nakajima, 1991).

2.4. Effective pK_a values

Effective pK_a values of oligochitosans were determined by potentiometric titration of oligochitosan hydrochlorides in accordance with the modified method published in Qin et al. (2006). Briefly, 50 ml of oligochitosan hydrochloride was dissolved (1 mg/ml) in distilled water and pH was adjusted by HCl to pH 3.00–3.05. The solution was titrated with 0.5 M NaOH while monitoring the solution pH. The equivalent inflexion points of titration curves were taken as pK_a of oligochitosan samples. All experiments were carried out in triplicate, and average values are shown in Table 1.

2.5. Dynamic light scattering (DLS)

DLS measurements were performed using PhotoCor Complex spectrometer (PhotoCor Instruments, Russia) equipped with pseudo cross-correlation system of photon counting and He–Ne laser as a light source (λ = 633 nm). The real-time correlator was employed in the logarithmic configuration. Measurements were performed in dilute solutions at 25 °C within the range of scattering angles of 30–140°. Distributions over decay time and hydrodynamic radius were obtained by means of CONTIN program. Apparent self-diffusion coefficients D were determined for each diffusive mode from angular dependence of the reciprocal relaxation time τ in accordance with the relation $D = 1/\tau q^2$, where $q = (4\pi n/\lambda)\sin(\theta/2)$ is wave vector. The corresponding hydrodynamic radii R_h were calculated from Stokes–Einstein relation:

$$R_h = \frac{kT}{6\pi\eta D}$$

where k is Boltzmann's constant, η is the solvent viscosity.

Sample solutions for DLS studies were prepared as follows: oligochitosan hydrochloride samples were dissolved in 0.1 M acetic acid (c = 3 mg/ml for sample 10, and 10 mg/ml for samples 3, 6 and 7), then the solution of 3 M tris(hydroxymethyl)aminomethane (TRIS) was added dropwise to oligochitosan solutions in order to adjust a desirable pH. Solutions were filtered through 0.22 μm pore-size Spartan nitrocellulose membranes. At critical pH value, 0.8 μm Millipore mixed cellulose ether membranes were used. No aging effect was observed in solutions except for the solutions corresponding to the critical points.

2.6. Transmission electron microscopy (TEM)

Transmission electron microscopy was performed with a LEO912 AB OMEGA electron microscope. The samples were prepared using 0.5–1% solution of oligochitosan hydrochlorides in 0.1 M acetic acid–TRIS buffer. A droplet of sample solution was deposited on Formvar-coated copper grid and dried for 1 min, then the excess of the solution was blotted off. The staining solution (1%

Table 1
Characteristics of oligochitosan samples.

Sample number	$M_w \pm 0.03$ (kDa)	PI ± 0.08	DP[a]	DD ± 1 (mol%)	p$K_a \pm 0.1$
1	0.73	1.41	4	95	7.1
2	1.52	1.39	8	93	6.7
3	2.09	1.40	12	97	6.6
4	3.58	1.71	20	95	6.5
5	4.22	1.38	24	97	6.5
6	6.40	1.56	39	97	6.5
7	9.69	1.44	56	97	6.4
8	12.80	1.39	74	95	6.4
9	15.10	1.61	87	94	6.4
10	20.00	1.66	116	98	6.4

[a]Average degree of polymerization was calculated in accordance with DD values.

solution of uranyl acetate) was added, blotted off, and dried in the air.

2.7. Bacterial strain and antibacterial test

Oligochitosan solutions were filtrated through a 0.22 μm pore-size syringe filter (Millipore, Swinnex) and stored at 4 °C until usage. The strain of methicillin-resistant *S. aureus* ATCC 35591 used in the studies was obtained from State Research Institute for Standardization and Control of Medical Biological Preparations (Moscow, Russia). The bacterial strain subcultured fortnightly was maintained on the plates with the standard B-medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% K_2HPO_4, 0.1% glucose, 12 g/l agar) at 4 °C and used as the starting inoculum culture. Bacteria stock solution (30 ml) was prepared by the use of 5% (v/v) inoculum.

2.8. Minimal inhibition concentration (MIC)

MICs of oligochitosan samples were determined by a broth microdilution assay in accordance with the method described in Raafat, Bargen, Haas, & Sahl (2008). Growth of the tested microorganism was monitored at 590 nm every 6 h for 48 h by an ELISA reader (Biolog, Hayward, CA). After 48 h the MIC was defined as the lowest concentration of oligochitosan required for complete inhibition of bacterial growth at the described conditions. Control experiments were carried out at different pH values without oligochitosan. The viable cells counts in control wells were determined after 48 h in order to define the influence of medium acidity on the bacterial growth. All experiments were carried out in triplicate.

3. Results and discussion

3.1. Oligochitosan preparation and characterization

In order to prepare the set of oligochitosan samples differing in M_w, we chose the most investigated method of depolymerization of HMW chitosan, namely the depolymerization of chitosan with hydrochloric acid. This method was chosen since (a) enzymatic method requires the usage of either an expensive purified form of chitosanolytic enzyme or a cheaper complex of chitosanolytic enzymes, and separation of oligochitosan from the enzymes (Aam et al., 2010; Ilyina, Tikhonov, Albulov, & Varlamov, 2000; Kim & Rajapakse, 2005). The M_w and activity of oligochitosan produced by enzymatic method depends on DD of the parent chitosan (Lin et al., 2009); (b) the impact of this method on the chemical structure of oligochitosan is minimal in comparison with the one that uses hydrogen peroxide (Qin, Du, & Xiao, 2002; Tian, Liu, Hu, & Zhao, 2004); (c) oligochitosan produced by acidic hydrolysis does not have reactive aldehyde end-groups capable of producing Schiff-bases and other by-products that are formed during the depolymerization of chitosan by means of nitrous acid (sodium nitrite) or periodic acid (sodium periodate) (Allan & Peyron, 1995; Tømmeraas, Vårum, Christensen, & Smidsød, 2007; Vold & Christensen, 2005).

The method of chitosan depolymerization in hydrochloric acid solution is highly specific, so that the cleavages between two conjunct *N*-acetylglucosamine units and between *N*-acelylglucosamine-glucosamine units mainly occurs (Einbu & Vårum, 2007; Einbu, Grasdalen, & Vårum, 2007).

The process of LMW chitosan depolymerization was carried out in hydrochloric acid at 70 °C for 1–6 h and led to the cleavage of glycosidic bonds and formation of oligochitosan of much lower M_w depending on the concentration of hydrochloric acid and on the treatment duration. After acidic hydrolysis, oligochitosan samples were analyzed by HP SEC method to determine their M_w and PI. The dependence of M_w on the time of acidic hydrolysis is shown in Fig. 1. Partial deacetylation was also observed, so that the degrees of deacetylation of oligochitosans obtained after the acidic hydrolysis (Table 1) were much higher (DD 94–99 mol%) than that of the parent chitosan in accordance with the previously published data (Einbu & Vårum, 2007; Vårum, Ottøy, & Smodsrød, 2001). As a result, a series of oligochitosan samples having M_w in the range of 0.73–20.00 kDa and a low polydispersity was obtained (Table 1).

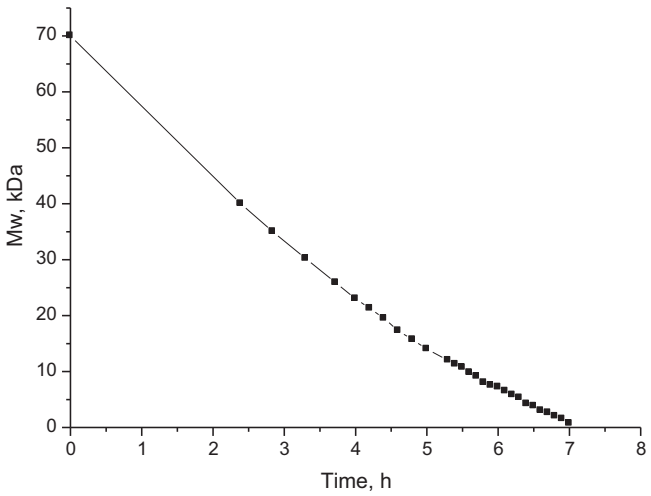

Fig. 1. LMW chitosan (70 kDa) hydrolysis in 0.6 M HCl at 70 °C.

3.2. Self-aggregation phenomenon

Like most natural polymers, chitosan has a tendency to self-aggregating in aqueous dilute and semidilute solutions. Thorough knowledge of self-aggregation of chitosan is highly significant for the accurate polymer characterization and practical application of chitosan-based materials since one can suppose that the self-assembly phenomenon may affect the biological properties of chitosan and oligochitosan. Most of the data on this phenomenon have been obtained for chitosan and its derivatives (Buhler, Guetta, & Rinaudo, 2000; Korchagina & Philippova, 2010; Philippova, Volkov, Sitnikova, & Khokhlov, 2001; Popa-Nita, Alcouffe, Rochas, David, & Domard, 2010; Sorlier, Rochas, Morfin, Viton, & Domard, 2003), while there is no information on the self-aggregation of unmodified oligochitosans in aqueous solutions. Here, it should be mentioned that although the boundaries between HMW, LMW, and oligochitosan are rather uncertain, from polymeric point of view chitosan which macromolecules have the average contour lengths comparable with persistence length (L_p) and Kuhn segment (A) or, in other words their DPs are comparable with that of L_p and A, may be referred to oligochitosan. The intrinsic persistence length of chitosan was determined in many works and its value was found in the range from 5 to 15 nm depending on the method of chitosan preparation, samples DD and acetyl-groups distribution along the chains, M_w of investigated sample, and method used for the evaluation of conformational parameters (Buhler et al., 2000; Lamarque, Lucas, Viton, & Domard, 2005; Rinaudo, Milas, & Dung, 1993; Schatz, Viton, Delair, Pichot, & Domard, 2003; Weinhold & Thoming, 2011). Taking into account that the length of monomeric unit equals 0.515 nm (Rinaudo et al., 1993), the length of the samples studied in our work correspond to the above mentioned values of L_p and A, and may be regarded as oligochitosans.

Several samples (samples 3, 6, 7, and 10) shown in Table 1 were studied by dynamic light scattering in dilute solutions (below c^*, as deduced from the intrinsic viscosity) as a function of pH. It is obvious from DLS data (Figs. 2 and 3) that in the whole pH range up to the critical values, which approximately coincide with pK_a

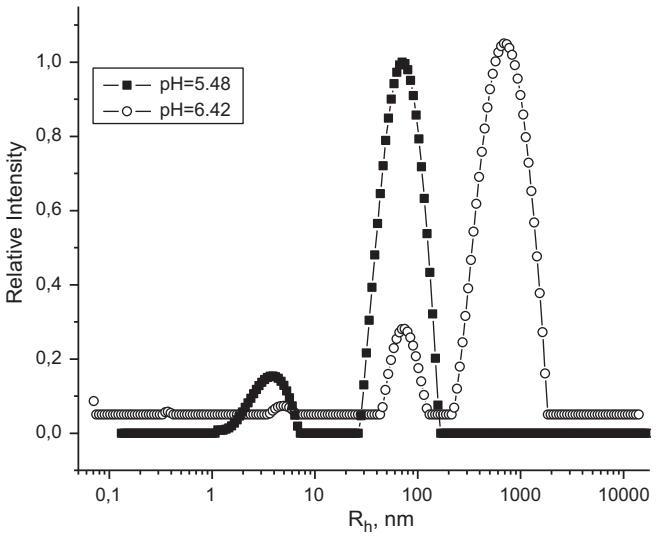

Fig. 2. Distribution of hydrodynamic radius of oligochitosan (sample 9) particles at pH below (5.48) and above (6.42) the critical value.

(Table 1), the main portion of scattered light is due to the diffusion motion of aggregates with hydrodynamic radii of about 40 nm for samples 3, 6, and, 7 and about 70 nm for sample 10. Hydrodynamic radii ($R_h^{(1)}$) of the fast diffusive mode agreed rather well with those calculated from SEC data. Due to this evidence, we can suppose that the observed aggregates are generated by rather weak bonds so that they are disrupted during dynamic experiments like SEC, which are characterized by a high shear rate at the top of the column as approximately $1 \times 10^4\ s^{-1}$. Indeed, rather narrow MWD curves without visible traces of large species in SEC experiments are obtained. Fig. 2 shows the variations of hydrodynamic radii of aggregates ($R_h^{(2)}$) as a function of pH depending on DP. As can be seen from this figure, the hydrodynamic radii of aggregates $R_h^{(2)}$ are almost independent of pH below its critical value despite the continuous variation in degree of dissociation up to approximately $\alpha = 0.5$ which is accompanied by an increase in ionic strength up to approximately 0.1 due to a partial neutralization of acetic acid. Above the critical pH value the macro-phase separation and the

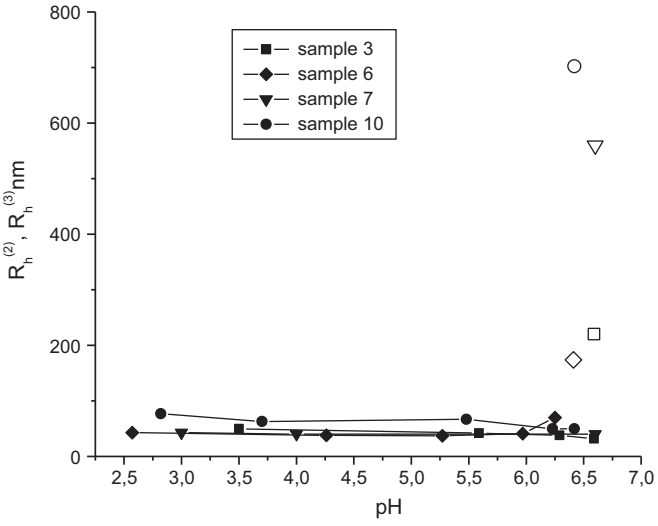

Fig. 3. The variation of hydrodynamic radius of primary aggregates ($R_h^{(2)}$, dark symbols) as a function of pH for chitosans of various DP and the sizes of secondary clusters ($R_h^{(3)}$, open symbols). For sample 7, R_h value at pH 6.47 corresponds to the mean value of two unresolved peaks of distribution curve.

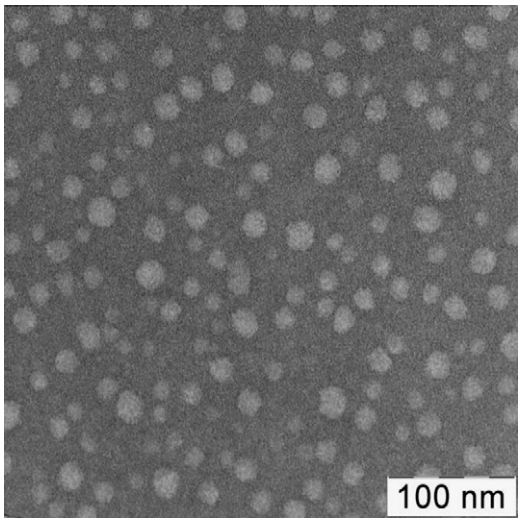

Fig. 4. TEM micrograph of multichain aggregates of oligochitosan (sample 3).

formation of gel-like precipitate are observed. At the critical pH values it is possible to observe the co-existence of two aggregation modes: primary aggregates ($R_h^{(2)}$) and their clusters ($R_h^{(3)}$) of the size approximately ten times as large (the example is shown in Fig. 2, pH 6.42). The inspection of angular dependences of DLS data confirms that each of these relaxation processes is diffusive with the diffusion coefficient equal to the slope of the graph $1/\tau$ vs q^2. The formation of self-aggregates in solutions of oligochitosan is also confirmed by TEM study of sample 3 (DP = 12). Fig. 4 shows the TEM image of aggregates of sample 3 whose solution was previously prepared for DLS studies. One can see that the sample contains quite well-defined particles of approximately spherical form with the diameter of about 20 nm. This result is rather surprising taking into account fairly short contour lengths of the chains which approximately coincide with the lowest estimated chitosan intrinsic persistent length. To the best of our knowledge, this is the first observation of two types of supramolecular particles (primery aggregates and clusters) coexisting in oligochitosan solutions. We can suppose in this connection that the primary aggregates existing in chitosan solutions may play a role of precursors of the sol/gel transition responsible for macro-phase separation in the dilute solutions and macroscopic gelation in more concentrated systems (Popa-Nita et al., 2010).

3.3. *Antibacterial activity of oligochitosan towards S. aureus (MRSA)*

From the practical point of view, oligochitosan has several advantages over chitosan: (a) reduced viscosity; (b) enchanced oral absorption; (c) compatibility with surfactants, stabilizers and emulsifiers, sugars, 40% ethanol, salt, glycerin, organic acids and colorants; (d) application of oligochitosan in pharmaceutical, textile, cosmetic and food products does not interfere with the current technologies used.

The published data concerning the biocidal activity of chitosan, oligochitosan and chitooligosaccharides against *S. aureus* contain the controversial results on the relationship between M_w and activity, and these data are as controversial as the data for other bacteria tested. Thus, HMW chitosan (M_w 28–1671 kDa) was found to be more active (MIC ≥ 800 μg/ml) in comparison with oligochitosan having M_w 1–22 kDa (DD values were not shown) (No, Park, Lee, & Meyers, 2002). Ultramicroscopic studies revealed that, chitooligosaccharides (M_w < 3 kDa, DD not shown) were less active then HMW chitosan (M_w 628 kDa, DD 80–85%) towards *S. aureus*

Table 2
MICs (μg/ml) of oligochitosan samples at different pH against *S. aureus* (MRSA). Initial bacteria concentration in control experiments was $2.5 \pm 0.5 \times 10^5$ CFU/ml.

pH	Control (without oligochitosan) (CFU/ml × 10^7)	M_w (kDa)								
		0.73	1.52	2.09	3.58	4.22	9.69	12.80	15.10	20.00
5.50	8.35	≥1000	≥1000	416	83	104	31	31	87	31
5.75	8.90	≥1000	≥1000	250	83	83	31	31	31	31
6.00	9.15	≥1000	≥1000	250	62	62	26	26	31	26
6.25	9.30	≥1000	≥1000	250	52	52	15	13	26	13
6.50	9.50	≥1000	≥1000	416	52	52	8	8	13	8
6.75	9.80	≥1000	≥1000	500	83	62	8	8	8	8
7.00	9.45	≥1000	≥1000	833	116	104	250	250	250	250
7.25	9.45	≥1000	≥1000	≥1000	250	208	500	500	250	500
7.50	9.40	≥1000	≥1000	≥1000	500	500	≥1000	≥1000	≥1000	≥1000
7.75	9.30	≥1000	≥1000	≥1000	833	833	≥1000	≥1000	≥1000	≥1000
8.00	9.00	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000	≥1000

The results are the averages of three experiments. The average deviations of experimental points are in the ranges ±25% of mean values.

(Eaton et al., 2008). Among chitooligosaccharides (M_w < 5 kDa; DD 80–85%) and HMW chitosans (M_w 107–628 kDa, DD 80–85%), the former ones exhibited the lowest activity. The MIC varied from 1000 μg/ml in the case of HMW chitosan to 2300 μg/ml in the case of chitooligosaccharides (Fernandes et al., 2008). The low molecular weight chitosan with the viscosity-average molecular weight ranged from 5 kDa to 27 kDa (DD 85%) showed a low activity (MIC ≥ 1000 μg/ml) towards *S. aureus* (Gerasimenko, Avdienko, Bannikova, Zueva, & Varlamov, 2004). The absence of the statistically reliable differences was found between three separate fractions of chitooligosaccharides M_w 10–5, 5–1 and <1 kDa (DD not shown). All these fractions had MIC 600 μg/ml for *S. aureus* (Jeon, Park, & Kim, 2001). The controversial data on the activity of a series of the well-defined chitosan samples (M_w 1.4–400 kDa, PI 1.49–4.12, DD 85–88%) against *S. aureus* was found in Qin et al. (2006) where it was shown that chitosan activity increased in the row 17 > 2.8 > 1.4 kDa and reduced in the row 78 > 17 > 48 > 130 > 400 kDa while half-*N*-acetylated chitosan and chitooligomers had no significant antimicrobial activity. No activity at all was found for enzymatically produced oligochitosan samples with M_w < 8 kDa, 10.1 kDa and 12.0 kDa although some samples with M_w 11–15 were active and had MIC 400–1600 μg/ml. Despite that in the agreement with the earlier found DD-activity relationship, antibacterial effect of active oligochitosan samples was higher in the case of samples with DD 92% than that of samples having DD 80% (Lin et al., 2009). Only once it was demonstrated that M_w of chitosan did not influence antibacterial activity of chitosan against *S. aureus* at all (Han & Jeon, 2004).

As reviewed above, in most cases oligochitosan samples were not characterized by PI and DD values, and only few oligochitosan samples were used in many of earlier studies. Therefore, there is a lack of data on biocidal activity of wide range of oligochitosan samples differing in their chain lengths against *S. aureus*, especially MRSA, and the basic question which one ought to clarify first of all is what molecular weight does oligochitosan has to have in order to possess the highest biocidal activity against *S. aureus* at neutral pH values?

As seen in Table 2, *S. aureus* cells growth significantly in pH range 5.5–8.0 while oligochitosans markedly inhibit the multiplication of MRSA cells, the effect dependent of pH value. Low bacteriostatic effects was observed if the complete inhibition of bacterial growth at the described conditions was not found even at 1000 μg/ml oligochitosan concentration. Oligochitosan samples 1 and 2 with the lowest M_ws (M_w 0.73 and 1.52 kDa) exhibit the lowest activity in pH ranged from 5.5 to 8.00. Their MIC values are found to be not less than 1000 μg/ml. Samples 4–10 (M_w 3.58–20.00 kDa) demonstrate the highest activity at pH values which are very close to neutral ones (pH 6.00–6.75). The activity reduces with the increase in pH since partial deprotonation of amino-groups of oligochitosan at basic pH leads to decrease in its bactericidal activity. Only samples 4 and 5 (M_w 3.58 and 4.22 kDa) possess moderate activity at neutral and basic pH 7.00–7.75. Nevertheless, the activity of samples 4–10 (M_w 3.58–20.00) is higher at pH 6.5 than that at pH 5.5 in contradiction to the published data (Wang, 1992). These results indicate that oligochitosans with M_w 3.58–20.00 (samples 4–9) can be used even at the neutral pH values. The enchanced oral absorption of oligochitosan having M_w 3–4 kDa (Chae, Jang, & Hah, 2005) let to consider it as a possible potential alternative for classic antibiotics both at neutral and basic pH values.

4. Conclusion

Depolymerization of LMW chitosan in hydrochloric acid leads to oligochitosan of low polydispersity and high degree of deacetylation. The antibacterial testing of oligochitosans obtained shows that oligochitosans having M_w in the range of 4–20 kDa can be used both at slightly acidic and neutral pH values, and that the activity against MRSA remains moderate for oligochitosan samples having M_w about 3–5 kDa even at slightly basic pH values. For the first time it is shown that oligochitosans, as well as HMW chitosan, form supramolecular aggregates in dilute solutions below their critical pH values which are near their pK_a. Despite the aggregation phenomenon, the formation of nano-sized aggregates does not prevent oligochitosan from demonstrating the antibacterial activity. In this paper we do not touch the mode of action of oligochitosan which has been determined and discussed in many referred papers but we suppose that the oligochitosan aggregates may play a role of precursors in further sol/gel transition above the critical pH values and may impact oligochitosan antibacterial activity. In our opinion, in order to appropriately recognize the mode of oligochitosan activity, multiple factors, such as MW, polydispersity, and self-assembling properties, should be considered.

Acknowledgements

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