

The effect of chitosan nanoparticles on *Escherichia coli* viability in drinking water disinfection

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ABSTRACT

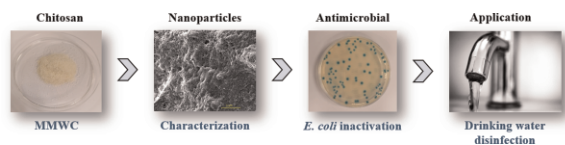
Drinking water disinfection or inactivation of pathogenic microorganisms is an essential step to minimize infection risks and decrease the incidence of waterborne diseases. Recently, chitosan nanoparticles (CNS) have been highlighted as an antimicrobial agent for a wide range of applications due to their natural antimicrobial properties, and low or no toxicity risk for human health. In this study, we generated CNS from three different molecular weight (MW) chitosan (low, medium, high) at various concentrations (0.25, 0.5 and 2% (w/v)). After the CNS preparation and quality assessment, the antimicrobial activity was evaluated by measuring the log reduction of Gram-negative bacteria *E. coli* as a model microorganism for faecal pollution. The results demonstrated that 0.25% of medium MW CNS are optimal for more than 99.99% reduction of cultivable *E. coli* and 97% inactivation of metabolically active *E. coli*. These results indicated that CNS were able to inhibit the growth of cultivable and metabolically active *E. coli* in tap water and demonstrated the potential use of CNS as an alternative antimicrobial agent in drinking water disinfection.

Key words: antimicrobial agent, chitosan, nanomaterials, water disinfection

HIGHLIGHTS

- CNS were synthesized by an ionic gelation method.
- MMWC with a chitosan concentration of 0.25% is the most suitable material for bacterial inactivation.
- In drinking water, chitosan nanoparticles inhibit both cultivable and metabolically active *E. coli*.

GRAPHICAL ABSTRACT



INTRODUCTION

The access to safe and reliable drinking water for everyone is the essential requirement to maintain healthy life. However, World Health Organization (WHO) estimates that by 2025, nearly half of the world's population will live in regions affected by water scarcity (WHO 2019). Therefore, there is an urgent need to provide technologies for production of clean and microbiologically safe drinking water in rural areas of both developed and developing regions. In this context, decentralized or point-of-use (POU) water treatment systems represent a sustainable way to treat water before consumption for private and domestic uses. Small-scale or POU drinking water systems can be used while travelling outdoors or in emergency situations when people are forced to consume unsafe water from untreated surface or groundwater sources (Patil *et al.* 2020). Today, with the rapid developments in nanotechnology, the production of non-toxic, environmentally safe and highly efficient nanoparticles suitable for water disinfection processes is becoming more and more widespread (Rikta 2019).

Currently, chitosan nanoparticles (CNS) have been receiving increasing attention due to their potential use in various industries ranging from food preservation and packaging (Priyadarshi & Rhim 2020), cosmetics (Chen

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et al. 2017), pharmaceutical applications (Andonegi *et al.* 2020) to agricultural areas (Jeon *et al.* 2016). These nanoparticles are regarded low toxicity, biocompatible and highly biodegradable (Kean & Thanou 2010). Chitosan is a linear polysaccharide consisting of randomly distributed β -(1 \rightarrow 4)-linked D-glucosamine and N-acetyl-D-glucosamine units and it is produced by deacetylation of chitin, which is extracted from the exoskeleton of crustaceans such as shrimps and crabs, as well from the cell walls of fungi, insects and yeasts. It is defined to be the second most widely distributed biopolymer in nature next to cellulose (1.5×10^{12} tons), with a production of approximately 10^{11} tons annually (Abidin *et al.* 2020). In addition to this, non-toxic and inherent antimicrobial properties make chitosan attractive for the use as an alternative antimicrobial agent in drinking water disinfection. There is a current trend for consumers to try to re-evaluate conventional disinfection methods, trying to reduce the amount of chemicals that can form toxic disinfection by-products (Mazhar *et al.* 2020). Thus, the application of chitosan in water treatment can be considered as a feasible and highly innovative approach with low or no toxicity to the consumers and the environment.

CNs, when compared to microparticles, are associated with a higher antimicrobial activity due to higher surface area to volume ratio and particle size effect. Perinelli *et al.* (2018) explained that the smaller particle size is associated with the higher activity due to larger contact area between the bacteria cell surface and particles. Therefore, parameters such as the size, shape and surface area are the key factors that determine the antimicrobial activity of nanoparticles. CNs, having a size from 10 to 100 nanometres, have been highlighted as antimicrobial agents in medicine (Javed *et al.* 2021), food (Garrido-Maestu *et al.* 2018) and water disinfection (Xiao *et al.* 2015) due to their natural antimicrobial properties, and low or no toxicity risk to human health. Currently many studies have evaluated the antimicrobial effect of CNs; however, most of them have been performed when CNs were used as stabilizing agent, composite material, filler, or base to develop new nanomaterials. As a result, the antimicrobial effect of CNs still remains hard to compare with previous studies.

In this paper, CNs have been investigated for their potential application as antimicrobial agents in drinking water disinfection. In addition, we hypothesize that the results of the present work will enable further research in the preparation and application of CNs in the drinking water treatment sector. Consequently, within this study CNs were prepared and evaluated for their potential application in neutralization of a Gram-negative water quality indicator microorganism – *Escherichia coli*.

MATERIALS AND METHODS

Preparation of chitosan nanoparticles

To evaluate the effect of molecular weight (MW), three different chitosan products were selected: low molecular weight (LMWC: 50,000–190,000 Da based on viscosity, 75–85% deacetylated, Sigma Aldrich, MO, USA); medium molecular weight (MMWC: 190,000–310,000 Da and 75–85% deacetylated, Sigma Aldrich, MO, USA) and high molecular weight (HMWC: 310,000–390,000 Da and degree of deacetylation $\geq 75\%$, Sigma Aldrich, MO, USA). Different concentrations of chitosan (0.25, 0.5 and 2% (w/v)) were selected to investigate the optimal chitosan concentration for chitosan nanoparticle (CN) preparation.

CNs were prepared by an ionic gelation method using the following standard protocol (Yang *et al.* 2015). Briefly, different concentrations of chitosan (0.25, 0.5 and 2% (w/v)) were added to a mixture of 2% acetic acid (v/v) and 1% Tween[®]80 (w/v). The solutions were stirred overnight with a constant shaking at 200 rpm until no particles were observed. For cross-linking of chitosan, 2 mL of 10% of sodium sulfate (SS, Sigma Aldrich, MO, USA) (w/v) were added to chitosan solutions under magnetic stirring (Biosan, Latvia) until the solutions became cloudy. Then, the solutions were sonicated for 20 min using a CPX 130 PB ultrasonic processor (Cole-Parmer Instruments, USA) with a sonication power of 60 W (50 Hz) to decrease their viscosity. After sonication, the nanoparticle suspensions were centrifuged at 8,500 rpm at 20 °C for 10 min (OHAUS, Germany). The pellets were re-suspended in sterile distilled water to wash the CNs and centrifuged again. Centrifuging and rinsing processes were repeated twice before freeze-drying of the pellets. The freeze-drying was performed using a Beta 2–8 LSC basic Laboratory Freeze Dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). The weight of CNs were measured after freeze-drying.

Characterization of CNs

The morphology of chitosan nanoparticle surface area was observed by scanning electron microscope (SEM, Tesnac MIRA\LMU, Brno, Czech Republic). The dried sample was put on a standard SEM aluminium pin

stub and then coated with a thin layer of gold (Emitech K550X, Quorum Technologies, Kent, United Kingdom) via a sputter coating technique. The sample surface morphologies were captured at an accelerating voltage of 30 kV and visualized at various magnifications. The specific surface area of the CNs were characterized by the Brunauer-Emmett-Teller (BET) technique based on physical nitrogen gas adsorption on a solid surface at an analysis temperature of -196°C . The analyses were performed using Quadrasorb SI (Quantachrome[®], USA) gas adsorption surface analyser at relative pressure (P/P_0) of 0.05.

Bacterial strains and culture conditions

Escherichia coli ATCC[®]25922 was used as an indicator microorganism to evaluate the disinfection efficiency. It was cultivated overnight in a sterile Luria-Bertani (LB) broth (10 g/L tryptone, 10 g/L NaCl, 5 g/L yeast extract, pH 7.0) at 37°C with constant shaking at 200 rpm (Biosan, Latvia). Then *E. coli* cells were thrice washed with centrifugation (6,000 rpm for 2 minutes, Minispin, Eppendorf) and rinsed with sterile phosphate buffered saline (PBS, 7 mM Na_2HPO_4 , 3 mM NaH_2PO_4 , 130 mM NaCl, pH 7.2) to remove the culture medium. The final bacterial pellet was re-suspended in sterile distilled water to obtain a stock solution of *E. coli* ($\sim 10^7$ CFU/mL). To determine the exact cell concentration, 0.005 mL of the stock suspension was filtered through a sterilized 25-mm diameter 0.2- μm pore size filter (Polycarbonate Track-Etch Membrane, Sartorius, Germany) and fixed with 3–4% formaldehyde for 10 minutes, washed with sterile distilled water and stained with 10 $\mu\text{g}/\text{mL}$ DAPI (4',6-diamidino-2-phenylindole, Merck, Germany) for 5–10 minutes. Cell concentration was determined with epifluorescence microscopy (Ex: 340/380; Em: >425 , dichromatic mirror 565 nm, Leica DM6000B, Germany) by counting of 20 random fields of view.

Disinfection tests

The disinfection tests were performed in pre-filtered tap water. The physico-chemical analysis of the tap water used for these experiments is given in Table 1 (Rigas *et al.* 2020). The total and free chlorine concentrations were below 0.05 mg/L Cl_2 (DR/890, HACH, USA). Approximately 5×10^5 CFU/mL of *E. coli* were inoculated into 2 mL of pre-filtered tap water (0.2 μm pore size filters) and mixed with CNs to final concentration of 3.5 mg/mL. Then the samples were incubated for 24 hours at 37°C with shaking at 200 rpm. Samples were collected after 0, 6 and 24 h. Each experiment was performed in triplicates. The sample without chitosan nanoparticles was used as a blank control.

Table 1 | The drinking water quality parameters in Riga, Latvia

Parameter	Value
<i>Escherichia coli</i>	0/100 mL
Coliform bacteria	0/100 mL
pH	7.4
Conductivity	485 $\mu\text{S}/\text{cm}$
Turbidity	0.2 NTU
Chloride	59 mg/L Cl^-
Sodium	31 mg/L
Iron	0.07 mg/L

Evaluation of *E. coli* inactivation

Cultivable cell counts. Cultivable *E. coli* was estimated with the plate count technique: 0.1 mL aliquot of the sample or its decimal dilution was inoculated on TBX agar (Oxoid Ltd, UK) and incubated for 24 hours at 37°C . Each sample was plated in triplicate. The results were expressed as colony forming units (CFU) per milliliter.

Cell respiratory activity measurements. The samples were incubated in equal amounts of sterile tryptone soya broth (TSB, Oxoid Ltd, UK) and 4 mM final concentration of CTC (5-cyano-2,3-ditolyl tetrazolium chloride, Fluka, BioChemika) for 1 hour in the dark at room temperature on an orbital shaker (200 rpm) and stained with DAPI as described before. Actively respiring and non-respiring *E. coli* cell numbers were determined with epifluorescence microscopy (Leica DM LB, Germany) for DAPI (Ex: 340/380; Em: >425) and for red fluorescent CTC-formazan crystals (Ex: $545 \text{ nm} \pm 30 \text{ nm}$; Em: $610 \text{ nm} \pm 75 \text{ nm}$). Metabolically active cells were determined by counting 60 random fields of view, giving a detection limit of 174 cells per mL.

Disinfection kinetics

Antibacterial activity of CNs against *E. coli* was calculated as Log reduction using the following equation (Equation (1)):

$$\text{Log reduction} = -\text{Log}_{10} (N/N_0) \quad (1)$$

where N_0 is the initial number of cultivable/metabolically active *E. coli* cells before disinfection experiment with CNs, N is the concentration of cultivable/metabolically active *E. coli* after disinfection with CNs at time t .

Statistical analysis

All experiments were performed in triplicates and the data were reported as the mean values with standard deviation (SD) from three separate experiments. Data were processed and visualized with PRISM 9 (GraphPad Software Inc., CA, USA).

RESULTS AND DISCUSSION

Characterization of CNs

The effect of the molecular weight of chitosan was tested, along with various concentrations; it was observed that MMWC with a chitosan concentration of 0.25% generated spherical nanoparticles (Figure 1(d)); however, when LMWC and HMWC were used no CNs were produced (Figure 1).

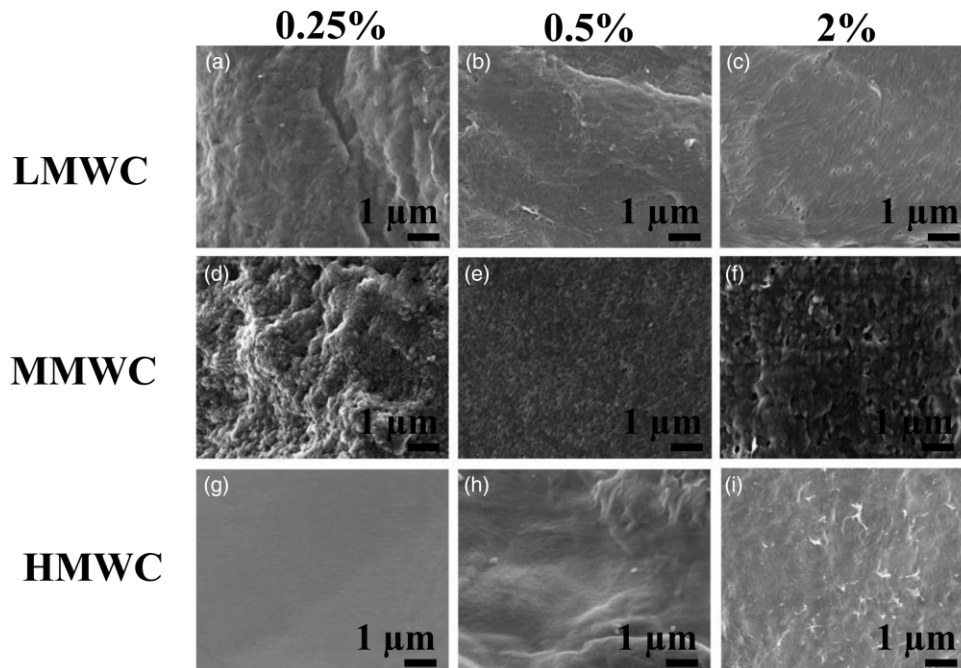


Figure 1 | SEM images of generated CNs using different MW chitosan and their various concentrations. Magnification $\times 10,000$ times.

The BET specific surface area was found to be $2.4 \text{ m}^2/\text{g}$ and $1.17 \text{ m}^2/\text{g}$ for 0.25% and 0.5% MMWC CNs respectively. According to Pivarciova *et al.* (2016), the value of specific surface area for chitosan medium molecular weight was $1.2 \text{ m}^2/\text{g}$. Therefore, the obtained results of 0.5% MMWC can be characterized as chitosan powder. This leads to the conclusion that the disinfection using the created CNs by 0.25% MMWC will be faster due to the increased specific surface area, which was also seen from the SEM images (Figure 1(d)). Therefore, according to the received results, 0.25% MMWC was selected as the optimal conditions for the preparation of CNs and were further applied to evaluate the antimicrobial properties against *E. coli*. However, the created CNs, using 0.25% MMWC, formed agglomerates; that is a common observation of conventional nanoparticle synthesis due to the mucoadhesive nature of chitosan (Sajomsang *et al.* 2011). The concentration of cross-linker, pH and chitosan concentration can significantly affect the formation of nanoparticles (Masarudin *et al.* 2015).

Similarly, CN aggregation can also occur after freeze-drying without any cryoprotectant agents due to intra- and intermolecular hydrogen bonds; as a result, their physical stability is affected (Abdelwahed *et al.* 2006). The formed agglomerates of CNs can have reduced antimicrobial properties due to the decrease of surface area. Alternatively, the agglomeration of CNs may show other favourable properties when compared to the single isolated nanoparticles due to their fractal structure. Therefore, it is essential to evaluate the antimicrobial effect of the agglomerated CNs to expand their applications.

Antimicrobial properties of CNs

To assess the applicability of generated CNs using 0.25% MMWC in drinking water disinfection, the antimicrobial activity was evaluated by measuring the log reduction of *E. coli*. The antimicrobial activity of the created CNs was evaluated at a final concentration of 3.5 mg/mL. The CNs concentration was selected based on previous studies (Garrido-Maestu *et al.* 2018) that have demonstrated that 0.2% was enough to completely inactivate food-borne pathogen *E. coli* O157:H7. However, within these studies the 0.2% CN showed the best antimicrobial activity in culture media and not in drinking water. The results of our studies indicated that CNs showed antimicrobial activity against cultivable and metabolically active *E. coli* cells in drinking water (Figure 2).

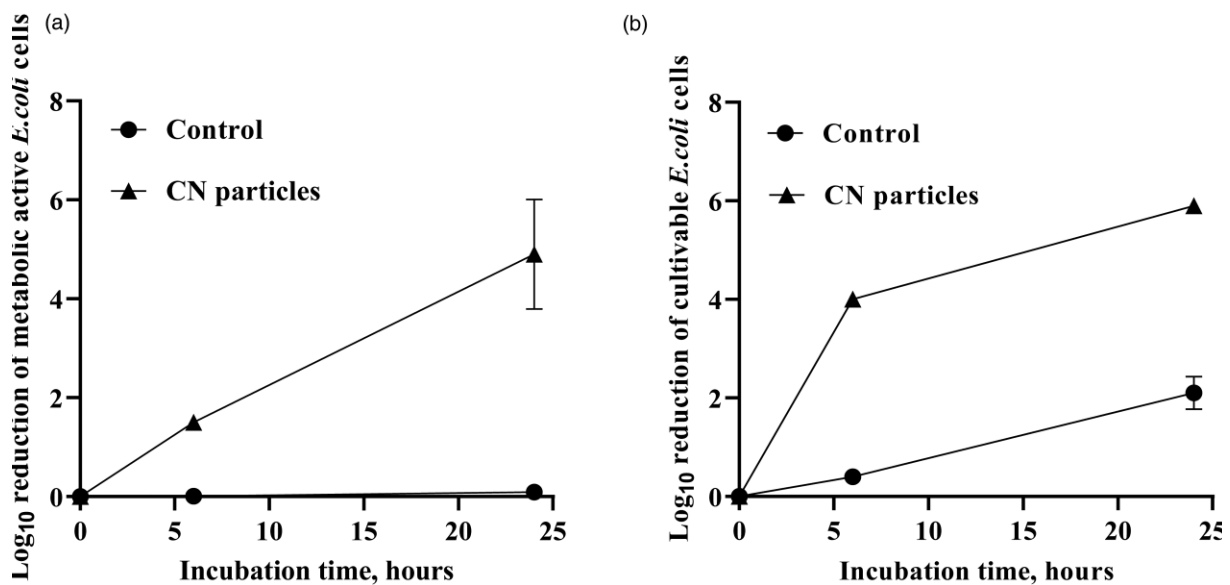


Figure 2 | Kinetics of antimicrobial activity of *E. coli* treated with CNs. CN generated with MMWC. (a) Log reduction of metabolically active *E. coli* cells. (b) Log reduction of cultivable *E. coli* cells. The data represent the average values of three separate experiments.

After the first 6 hours, more than 99.99% (4.0 ± 0.06 log) reduction in cultivable *E. coli* count was obtained with the tested CNs (Figure 2(b)). At the same time, the metabolic activity of *E. coli* decreased less, showing a decline of only 1.5 ± 0.11 log (97% inactivation) (Figure 2(a)) after 6 hours. When the contact time was prolonged to 24 hours of treatment, a 5.9 ± 0.09 and 4.9 ± 1.1 log reduction in cultivable and metabolically active *E. coli* were obtained, respectively. At the same time, in control samples 0.1 ± 0.01 and 2.1 ± 0.33 log reduction for metabolically active and cultivable *E. coli* were obtained within 24 h (Figure 2). The results of our study demonstrated that the inactivation activity was highly dependent on the longer contact time. It is apparent that *E. coli* could survive at higher CN concentration and longer period than was previously reported when alternative viability markers than cultivation are assessed.

Comparison of the results from different research is still complicated due to various CN producing methods, and testing under diverse conditions (Ma *et al.* 2017). The minimum inhibitory concentration (MIC) values of chitosan nanoparticles vary considerably, depending on the bacterial strain examined and on its growth phase; furthermore, the type of chitosan, its molecular weight, degree of deacetylation, temperature, pH and medium composition are crucial (Sarwar *et al.* 2014). For Gram-negative bacteria, the MIC values ranged from 0.00025 to 40 mg/mL. (Du *et al.* 2009) reported that the MIC value of unloaded CNs made with

tripolyphosphate (TPP) against *E. coli* was 0.117 mg/mL. Interestingly, Qi *et al.* (2004) also tested the MIC of CNs made with TPP against *E. coli*, *S. choleraesuis*, *S. typhimurium* and *S. aureus*; it was found that chitosan nanoparticles with MIC values less than 0.00025 mg/mL can inhibit the growth of these microorganisms. Another study showed that the MIC of CNs was 40 mg/mL against *E. coli* (Divya *et al.* 2017). As a result, comparison of CN efficacy between different studies is problematic since previous works from the literature have used chitosan with different molecular weight and degree of deacetylation (DA), and various methods have been used to produce CNs. Therefore, prior to technical introduction of CN as disinfecting agent validation and set up of crucial parameters; for example the effect of surface area, pH influence and used concentration is essential. In addition, separation of nanoparticles from the treated water should be introduced to promote effective and reliable POU drinking water treatment systems with no risks on human health and environmental safety.

Furthermore, many microorganisms, when subjected to stress, may be present in drinking water in a viable but noncultivable (VBNC) state (Ye *et al.* 2020), where they cannot be grown on traditional culture media. Therefore, to evaluate the disinfection kinetics of novel compounds, alternative viability forms of waterborne microorganisms should be considered. Chitosan nanoparticles have demonstrated a higher antibacterial effect than chitosan solution due to the higher density of the positively charged amino groups to the negatively charged surface of bacteria (Perinelli *et al.* 2018). This interaction resulted in cell membrane damage and caused the release of intracellular contents, and, as a result, cell death (Chung *et al.* 2004). Recently, Kritchenkov *et al.* (2019) have investigated the antimicrobial activity of new water-soluble chitosan derivatives and their nanoparticles prepared by ultrasound-assisted catalyst-free phenol-yne reaction, to enhance antimicrobial activity and broaden their applicability in different fields. The antimicrobial activity within the present study was determined by both *E. coli* cell cultivability and metabolic activity at a cellular level via assessing the functionality of the electron transport chain. The results indicated that CNs more rapidly inactivated *E. coli* ability to divide and only then their metabolic activity; for example, the functionality of the electron transport chain. Nevertheless, after a certain time, CN could reduce the overall activity of cells and demonstrate the potential of CN in environments with apparent presence of unculturable and stressed cells.

CONCLUSIONS

Chitosan nanoparticles were successfully prepared using the ionic gelation method. MMWC with a final concentration of 0.25% chitosan provides the best conditions for production of chitosan nanoparticles with sufficient antimicrobial efficacy. In addition, 3.5 mg/mL CNs can inhibit both cultivable and metabolically active *E. coli* in drinking water. The results of the current work indicate that the prepared CNs are a potential antibacterial material that can be applied as a natural and consumer-safe bactericidal agent for drinking water disinfection, in designing point-of-use drinking water treatment systems.

ACKNOWLEDGEMENTS

This research work was supported by ERA-NET EU-LAC Health project Efficient and affordable water treatment technologies to minimize waterborne diseases, Hi-Water (No. ES RTD/2018/26); and Riga Technical University's Doctoral Grant programme (DOK.BIF/20). The authors are grateful to Kristaps Rubenis of Institute of General Chemical Engineering for technical assistance, and Rudolfs Cimdins Riga Biomaterials Innovation and Development Centre of Riga Technical University for allowing use of their equipment for this research.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

- Abdelwahed, W., Degobert, G., Stainmesse, S. & Fessi, H. 2006 Freeze-drying of nanoparticles: formulation, process and storage considerations. *Advanced Drug Delivery Reviews* **58**(15), 1688–1713.
- Abidin, N. A. Z., Kormin, F., Abidin, N. A. Z., Anuar, N. A. F. M. & Bakar, M. F. A. 2020 The potential of insects as alternative sources of chitin: an overview on the chemical method of extraction from various sources. *International Journal of Molecular Sciences* **21**(14), 1–25.
- Andonegi, M., Heras, K. L., Santos-Vizcaíno, E., Igartua, M., Hernandez, R. M., de la Caba, K. & Guerrero, P. 2020 Structure-properties relationship of chitosan/collagen films with potential for biomedical applications. *Carbohydrate Polymers* **237**(March), 116159.

- Chen, K., Guo, B. & Luo, J. 2017 Quaternized carboxymethyl chitosan/organic montmorillonite nanocomposite as a novel cosmetic ingredient against skin aging. *Carbohydrate Polymers* **173**, 100–106. <http://dx.doi.org/10.1016/j.carbpol.2017.05.088>.
- Chung, Y. C., Su, Y. P., Chen, C. C., Jia, G., Wang, H. I., Wu, J. C. G. & Lin, J. G. 2004 Relationship between antibacterial activity of chitosan and surface characteristics of cell wall. *Acta Pharmacologica Sinica* **25**(7), 932–936.
- Divya, K., Vijayan, S., George, T. K. & Jisha, M. S. 2017 Antimicrobial properties of chitosan nanoparticles: mode of action and factors affecting activity. *Fibers and Polymers* **18**, 221–230.
- Du, W. L., Niu, S. S., Xu, Y. L., Xu, Z. R. & Fan, C. L. 2009 Antibacterial activity of chitosan tripolyphosphate nanoparticles loaded with various metal ions. *Carbohydrate Polymers* **75**(3), 385–389. <http://dx.doi.org/10.1016/j.carbpol.2008.07.039>.
- Garrido-Maestu, A., Ma, Z., Paik, S. Y. R., Chen, N., Ko, S., Tong, Z. & Jeong, K. C. C. 2018 Engineering of chitosan-derived nanoparticles to enhance antimicrobial activity against foodborne pathogen *Escherichia coli* O157:H7. *Carbohydrate Polymers* **197**(June), 623–630.
- Javed, R., Rais, F., Kaleem, M., Jamil, B., Ahmad, M. A., Yu, T., Qureshi, S. W. & Ao, Q. 2021 Chitosan capping of CuO nanoparticles: facile chemical preparation, biological analysis, and applications in dentistry. *International Journal of Biological Macromolecules* **167**, 1452–1467.
- Jeon, S. J., Ma, Z., Kang, M., Galvão, K. N. & Jeong, K. C. 2016 Application of chitosan microparticles for treatment of metritis and in vivo evaluation of broad spectrum antimicrobial activity in cow uteri. *Biomaterials* **110**, 71–80.
- Kean, T. & Thanou, M. 2010 Biodegradation, biodistribution and toxicity of chitosan. *Advanced Drug Delivery Reviews* **62**(1), 3–11. <http://dx.doi.org/10.1016/j.addr.2009.09.004>.
- Kritchenkov, A. S., Egorov, A. R., Volkova, O. V., Kritchenkov, I. S., Kurliuk, A. V., Shakola, T. V. & Khrustalev, V. N. 2019 Ultrasound-assisted catalyst-free phenol-yne reaction for the synthesis of new water-soluble chitosan derivatives and their nanoparticles with enhanced antibacterial properties. *International Journal of Biological Macromolecules* **139**, 103–113.
- Ma, Z., Garrido-Maestu, A. & Jeong, K. C. 2017 Application, mode of action, and in vivo activity of chitosan and its micro- and nanoparticles as antimicrobial agents: a review. *Carbohydrate Polymers* **176**(July), 257–265.
- Masarudin, M. J., Cutts, S. M., Evison, B. J., Phillips, D. R. & Pigram, P. J. 2015 Factors determining the stability, size distribution, and cellular accumulation of small, monodisperse chitosan nanoparticles as candidate vectors for anticancer drug delivery: application to the passive encapsulation of [¹⁴C]-doxorubicin. *Nanotechnology, Science and Applications* **8**, 67–80.
- Mazhar, M. A., Khan, N. A., Ahmed, S., Khan, A. H., Hussain, A., Rahisuddin, Changani, F., Yousefi, M., Ahmadi, S. & Vambol, V. 2020 Chlorination disinfection by-products in municipal drinking water – a review. *Journal of Cleaner Production* **273**, 123159.
- Patil, R., Ahmad, D., Balkundae, P., Kausley, S. & Malhotra, C. 2020 Development of low cost point-of-use (POU) interventions for instant decontamination of drinking water in developing countries. *Journal of Water Process Engineering* **37**, 101435.
- Perinelli, D. R., Fagioli, L., Campana, R., Lam, J. K. W., Baffone, W., Palmieri, G. F., Casettari, L. & Bonacucina, G. 2018 Chitosan-based nanosystems and their exploited antimicrobial activity. *European Journal of Pharmaceutical Sciences* **117**(January), 8–20.
- Pivarciova, L., Rosskopfova, O., Galambos, M., Rajec, P. & Hudec, P. 2016 Sorption of pertechnetate anions on chitosan. *Journal of Radioanalytical and Nuclear Chemistry* **308**, 93–98.
- Priyadarshi, R. & Rhim, J.-W. 2020 Chitosan-based biodegradable functional films for food packaging applications. *Innovative Food Science & Emerging Technologies* **62**, 102346.
- Qi, L., Xu, Z., Jiang, X., Hu, C. & Zou, X. 2004 Preparation and antibacterial activity of chitosan nanoparticles. *Carbohydrate Research* **339**(16), 2693–2700.
- Rigas udens 2020 Mean Parameters of Drinking Water Quality in the Central Water Distribution Network of Riga City (Cited December 15th 2020).
- Rikta, S. Y. 2019 9 – Application of nanoparticles for disinfection and microbial control of water and wastewater. In: Nanotechnology in Water and Wastewater Treatment *Theory and Applications*. *Micro and Nano Technologies*. Elsevier, Amsterdam, the Netherlands, pp.159–176.
- Sajomsang, W., Gonil, P., Ruktanonchai, U. R., Pimpha, N., Sramala, I., Nuchuchua, O., Saesoo, S., Chaleawler-umpon, S. & Puttipipatkachorn, S. 2011 Self-aggregates formation and mucoadhesive property of water-soluble β -cyclodextrin grafted with chitosan. *International Journal of Biological Macromolecules* **48**(4), 589–595.
- Sarwar, A., Katas, H. & Zin, N. M. 2014 Antibacterial effects of chitosan-tripolyphosphate nanoparticles: impact of particle size molecular weight. *Journal of Nanoparticle Research* **16**(7), 2517.
- WHO, World Health Organisation, Drinking water 2019. Available from: <https://www.who.int/news-room/fact-sheets/detail/drinking-water> (accessed 9 October 2021).
- Xiao, G., Zhang, X., Zhang, W., Zhang, S., Su, H. & Tan, T. 2015 Visible-light-mediated synergistic photocatalytic antimicrobial effects and mechanism of Ag-nanoparticles@chitosan-TiO₂ organic-inorganic composites for water disinfection. *Applied Catalysis B: Environmental* **170–171**, 255–262. <http://dx.doi.org/10.1016/j.apcatb.2015.01.042>.
- Yang, J., Han, S., Zheng, H., Dong, H. & Liu, J. 2015 Preparation and application of micro/nanoparticles based on natural polysaccharides. *Carbohydrate Polymers* **123**, 53–66.
- Ye, C., Lin, H., Zhang, M., Chen, S. & Yu, X. 2020 Characterization and potential mechanisms of highly antibiotic tolerant VBNC *Escherichia coli* induced by low level chlorination. *Scientific Reports* **10**(1), 1–11.

First received 21 July 2021; accepted in revised form 28 January 2022. Available online 9 February 2022