SYNTHESIS AND PHYSICOCHEMICAL EVALUATION OF NEW CHITOSAN-BASED SCAFFOLDS FOR POTENTIAL APPLICATION IN BIOMEDICINE

Martina Žabčić[1], Nataša Nikolić[1], Dušan Mijin[1], Zorica Kačarević Popović[2], Slavica Porobić[2], Julijana Tadić[2,*]

[1]University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4, Belgrade, Serbia
[2]University of Belgrade, Vinča Institute of Nuclear Sciences, National Institute of the Republic of Serbia, Mike Petrovića Alasa 12-14, Belgrade, Serbia
e-mail: julijana.tadic@vin.bg.ac.rs

Abstract
Chitosan is a natural polymer obtained by deacetylation of chitin. Due to its biocompatibility and biodegradability, chitosan-based materials have a wide range of biomedical applications in wound dressings, drug delivery systems, and tissue engineering. Also, studies in the fields of organic and medicinal chemistry show that compounds based on the pyridone core exhibit biological properties including antimicrobial, anticancer, and antioxidant activity, and moreover have the potential as new therapeutics for various diseases from cardiovascular to antitumor therapy. In this study, new biomaterials were synthesized using low and medium molecular weight chitosan polymers and pyridone-based hydrazone. In order to improve stability of the obtained scaffolds, scaffolds' neutralization was carried out using ethanol and sodium hydroxide solutions. The interactions established between chitosan polymer chains and pyridone compound were analyzed by FT-IR spectroscopy. Swelling and degradation tests of the materials were studied in water and PBS, and the influence of different polymer molecular weights on the scaffolds' properties was evaluated. The results indicated that synthetized scaffolds have a high potential for biomedical use.

Introduction
Chitosan is a linear polysaccharide consisted of D-glucosamine and N-acetyl-D-glucosamine units connected by the β-(1-4) glycosidic bond. This polysaccharide shows antibacterial, antifungal, analgesic, and antioxidant activity, hemostatic properties, and excellent biocompatibility [1,2]. All these unique characteristics make chitosan an outstanding candidate for biomedical applications. Development of biomaterials is a very attractive research field aiming to design scaffolds for the regeneration of tissues and organs damaged by disease or injuries [3]. Today, chitosan-based biomaterials have major applications in tissue engineering due to their biocompatibility and therapeutic properties [4]. Pyridone-based arylhydrazones have gained attention in the fields of biomedicine and bioimaging regarding their both biological and optical properties [5-7]. Furthermore, they exhibit antibacterial, antifungal, antioxidant, analgesic, anti-inflammatory, antitubercular and anticancer properties [8]. Taking that into account, in this study, we synthetized new scaffolds using chitosan and pyridone-based arylhydrazones, and evaluated their physicochemical properties important for potential application in biomedical research.

Experimental
Low molecular weight chitosan (50-190 kDa) and medium molecular weight chitosan (190-310 kDa), HIT-LW and HIT-MW, hereinafter, were purchased from Merck, and pyridone-based arylhydrazone was synthetized and characterized in our previous study [9]. In order to prepare

the scaffolds, HIT-LW and HIT-MW were dissolved in the acetic acid (2% v/v) to obtain 2% w/v solutions of HIT-LW and HIT-MW. Prepared chitosan solutions were stirred with heating at 50°C for 3 hours. Previously synthesized pyridone-based compound was dissolved in ethanol (4 mg in 5 ml of EtOH) and added to each HIT-LW and HIT-MW solution. Obtained mixtures were stirred with heating at 50°C for 1 hour. Resulting mixtures HIT-LW-PY and HIT-MW-PY were poured into the Petri dishes and left at -18°C for 24 hours. Frozen mixtures of HIT-LW-PY and HIT-MW-PY were freeze-dried for 48 hours, at a temperature of -50°C and a pressure (~0.1 mbar) to obtain 3D porous scaffolds. Along with the synthesis of new scaffolds, control materials were also synthesized, by freeze-drying HIT-LW and HIT-MW without addition of pyridone. The stabilization of prepared scaffolds was done by using NaOH and EtOH. The materials were immersed in 0.25 M NaOH solution, and in absolute ethanol and 70% ethanol, washed with distilled water, frozen and freeze-dried again. Fourier transform infrared (FT-IR) spectra of the scaffolds were recorded using a Nicolet™ iS™ 10 FT-IR Spectrometer (Thermo Fisher Scientific) with Smart iTR™ Attenuated Total Reflectance (ATR) sampling accessories. The FT-IR spectra were recorded in the 4000-500 cm−1 range with 20 scans per spectrum.

The swelling properties were determined by the conventional gravimetric method [10]. The chitosan-based scaffolds (disc; diameter 20 mm, thickness 0.5 mm) were weighed (W_0) and then immersed in water and weighed in specific time periods (W_s) until constant weight was reached. The equilibrium swelling degree was calculated using equation $SD_{eq} = \frac{W_s - W_0}{W_0}$.

The *in vitro* degradation was tested after incubation of 28 days in PBS at 37˚C [10]. Initial weights of the materials were obtained as W_0. After immersion, the scaffolds were washed in distilled water, frozen and freeze-dried, and then weighted and labeled as W_d. The degradation of the scaffolds (D) was calculated using equation $D(\%) = \frac{W_0 - W_d}{W_0} \cdot 100$.

Results and discussion

The interactions established between scaffolds' components were evaluated by FT-IR spectroscopy. The spectra of the synthetized scaffolds and control samples are shown in Fig. 1. In Fig. 1 A), the spectra of the prepared scaffolds after neutralization in ethanol are presented. The spectra of control scaffolds showed characteristic bands in the regions of 3000–3600 cm^{-1} (–OH and –NH groups of chitosan) and 2800–3000 cm^{-1} (CH groups). The bands at 1652 cm^{-1} and 1563 cm^{-1} were assigned to the amide I and amide II groups. The peak at 1153 cm^{-1} indicated the stretching vibrations of the C–O–C bonds, while the peaks at 1068 cm^{-1} and 1026 cm^{-1} corresponded to the vibrations of the C–O bonds [10]. The spectra of new scaffolds showed the characteristic bands for chitosan polymer including some changes in the intensity of the band in the region of 3000–3600 cm^{-1} indicating hydrogen bonding between chitosan and pyridone-based arylhydrazone. In Fig. 1 B), the spectra of scaffolds stabilized in sodium hydroxide showed same characteristic peaks as the spectra of materials stabilized in ethanol indicating that method of neutralization, i.e., stabilization of the material did not affect the material chemical structure. The hydrogen bonding of chitosan and pyridone-based compound is illustrated in Fig. 2.

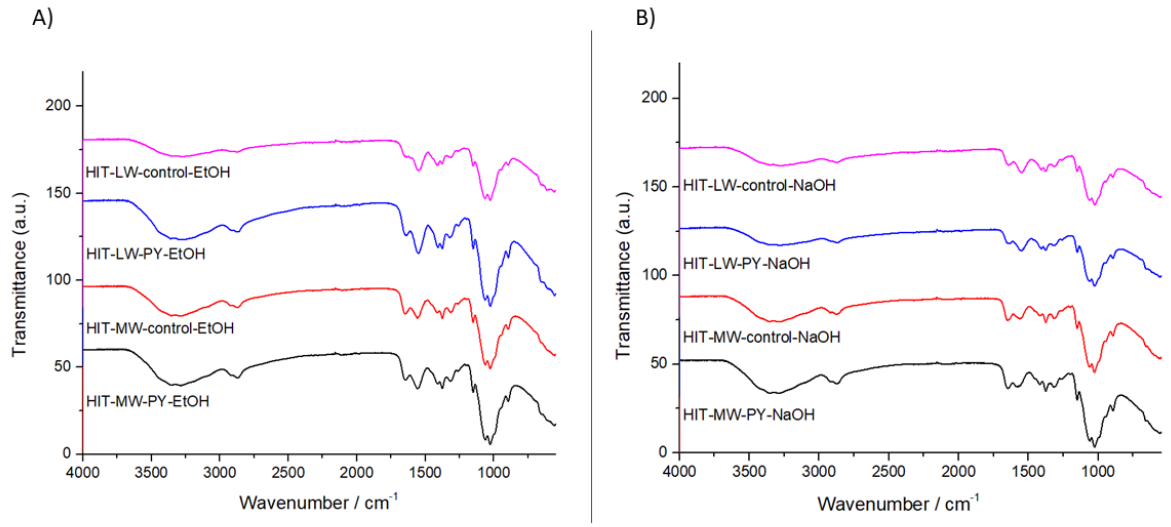

Figure 1. FT-IR spectra of new scaffolds and control samples A) stabilized in EtOH and B) stabilized in NaOH

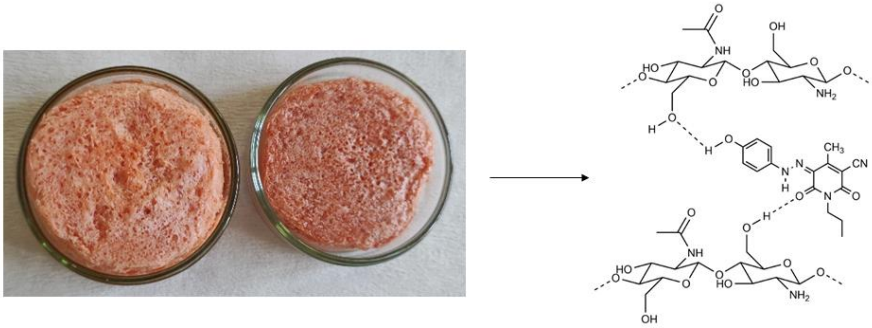

Figure 2. The illustration of synthetized scaffolds and the hydrogen bonding of the chitosan and pyridone-based arylhydrazone

From the standpoint of biomedical application, adequate swelling ability, porosity and degradation are very important factors in scaffold design. The swelling properties of the scaffolds was evaluated in water for 300 minutes, and obtained results are presented in Fig. 3. HIT-LW-PY-EtOH and HIT-MW-PY-EtOH scaffolds swelled very rapidly absorbing a large amount of water, and reaching the equilibrium swelling degree within initial 30 minutes (Fig. 3 A)). The scaffolds HIT-LW-PY-NaOH and HIT-MW-PY-NaOH swelled fast reaching the equilibrium swelling degree, also, within first 30 minutes (Fig. 3 B)). By comparing the HIT-LW-PY-EtOH and HIT-MW-PY-EtOH to the HIT-LW-PY-NaOH and HIT-MW-PY-NaOH, it can be noted that scaffolds stabilized in sodium hydroxide have a lower value of the equilibrium swelling degree (Fig. 3 C)) which may be attributed to their pore size (less porous comparing to the those stabilized in ethanol) obtained after stabilization. Also, the influence of different molecular weights of chitosan polymer on the equilibrium swelling degree was more pronounced in case of the scaffolds obtained by the stabilization in sodium hydroxide. Namely, HIT-MW-PY-NaOH has about a 50% higher value of the equilibrium swelling degree comparing to HIT-LW-PY-NaOH, which can generally be ascribed to the larger and more crosslinked medium molecular weight chitosan chains leading to a greater expansion of the

scaffold's network. However, it is clear that materials treated in ethanol have a higher absorption capacity so the stabilization method affected the scaffolds swelling properties.

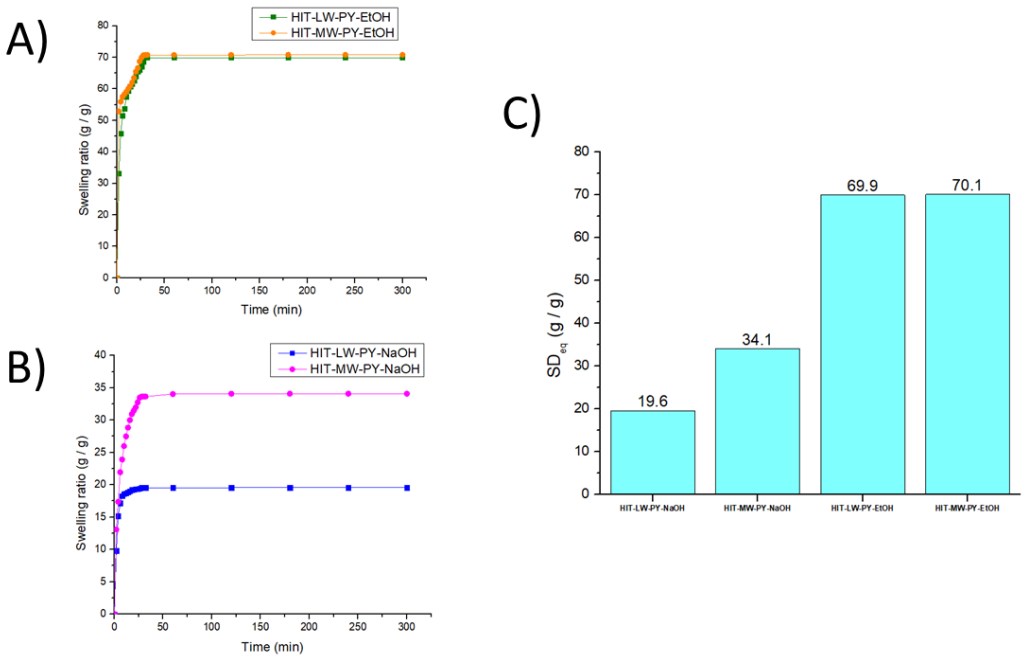

Figure 3. Swelling test in water

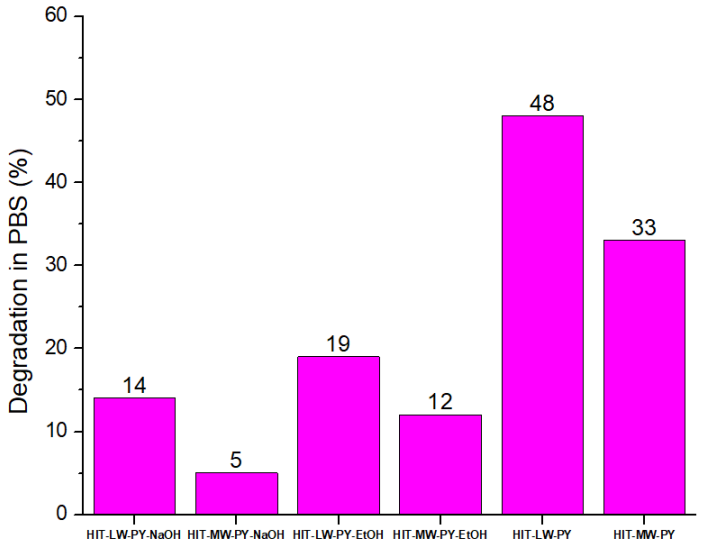

Figure 4. Degradation test in PBS

The degradation properties of the scaffolds were investigated in PBS after 28 days, and obtained results pointed out that molecular mass of chitosan and the method of stabilization affect the degradation degree of synthesized materials (Fig. 4). The scaffolds consisted of the low molecular weight chitosan had a higher degradation degree comparing to the those synthetized of the medium molecular weight chitosan. Also, materials stabilized in sodium hydroxide showed lower degradation in PBS compared to the materials stabilized in ethanol. Contrary, scaffolds that are not stabilized exhibited a significantly higher degradation in PBS indicating the importance of scaffold stabilization for further use.

Conclusion

In this work, new chitosan-pyridone-based scaffolds were synthesized and characterized. The structure of scaffolds and the formation of hydrogen bonds, between chitosan and pyridone derivative, were confirmed and observed by FT-IR spectroscopy. The swelling and degradation

tests in water and PBS highlighted that the scaffolds stabilized in ethanol exhibited adequate physicochemical properties and have the potential for further development and application in biomedical research field.

Acknowledgements
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References
[1] F. Croisier, C. Jérôme, European Polymer Journal 49 (2013) 780-792.
[2] F. Luan, L. Wei, J. Zhang, W. Tan, Y. Chen, F. Dong, Q. Li, Z. Guo, Molecules 23 (2018) 516
[3] B. Sultankulov, D. Berillo, K. Sultankulova, T. Tokay, A. Saparov, Biomolecules 9 (2019) 470
[4] A. Madni, R. Kousar, N. Naeema, F. Wahid, Journal of Bioresources and Bioproducts 6 (2021) 11-25
[5] X. Ran, Q. Zhou, J. Zhang, S. Wang, G. Wang, H. Yang, X. Liu, Z. Wang, X. Yu, Organic Chemistry Frontiers 8 (2021) 3631-3638
[6] Y. Ali, S. A. Hamid, U. Rashid, Mini-Reviews in Medicinal Chemistry 18 (2018) 1548-1558
[7] H. Ur R. Shaha, K. Ahmada, H. A. Naseema, S. Parveena, M. Ashfaqa, T. Aziz, S. Shaheena, A. Babras, A. Shahzad, Journal of Molecular Structure 1244 (2021) 131181
[8] J. D. Tadić, J. M. Lađarević, Ž. J. Vitnik, V. D. Vitnik, T. P. Stanojković, I. Z. Matić, D. Ž. Mijin, Dyes and Pigments 187 (2021) 109123
[9] J. Tadić, J. Lađarević, M. Svetozarević, L. Matović, A. Mašulović, D. Mijin, Conference proceedings, ISBN 978-86-85535-08-6, 34, Procesing ’21, Jun 3-4, 2021, Novi Sad, Serbia.
[10] J. Hu, Z. Wang, J. M. Miszuk, M. Zhu, T. I. Lansakara, A. V. Tivanski, J. A. Banas, H. Sun, Carbohydrate Polymers, 271 (2021) 118440.