MME SEE CONGRESS 2023

5th Metallurgical & Materials Engineering Congress of South-East Europe
Trebinje, Bosnia and Herzegovina
7-10th June 2023

CONGRESS PROCEEDINGS

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The Association of Metallurgical Engineers of Serbia

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Institute for Technology of Nuclear and Other Mineral Raw Materials in Belgrade, Serbia;
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The Faculty of Technology at the University of Banja Luka, Bosnia and Herzegovina;
The Faculty of Metallurgy at the University of Zagreb in Sisak, Croatia;
The Faculty of Natural Sciences and Engineering at the University of Ljubljana, Slovenia;
The Faculty of metallurgy and technology at the University of Podgorica, Montenegro.

CONGRESS PROCEEDINGS - MME SEE 2023
5th Metallurgical & Materials Engineering Congress of South-East Europe

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PREFACE

On behalf of the Scientific and Organizing Committee, it is a great honor and pleasure to wish all the participants a warm welcome to the Fifth Metallurgical & Materials Engineering Congress of South-East Europe (MME SEE 2023) which is being held in Trebinje, Bosnia and Hercegovina, 07 - 10 June 2023.

The MME SEE 2023 is a biannual meeting of scientists, professionals, and specialists working in the fields of metallurgical and materials engineering. The aim of the Congress is to present current research results related to processing/structure/property relationships, advances in processing, characterization, and applications of modern materials. Congress encompasses a wide range of related topics and presents the current views from both academia and industry: Future of metals/materials industry in South-East European countries; Raw materials; New industrial achievements, developments and trends in metals/materials; Ferrous and nonferrous metals production; Metal forming, casting, refractories and powder metallurgy; New and advanced ceramics, polymers, and composites; Characterization and structure of materials; Recycling and waste minimization; Corrosion, coating, and protection of materials; Process control and modeling; Nanotechnology; Sustainable development; Welding; Environmental protection; Education; Accreditation & certification.

The editors hope that Congress will stimulate new ideas and improve knowledge in the field of metallurgical and materials engineering. The Congress has been organized by the Association of Metallurgical Engineers of Serbia, with the co-organization of the Institute for Technology of Nuclear and Other Mineral Raw Materials, Belgrade, Serbia, Faculty of Technology and Metallurgy, University of Belgrade, Serbia, Faculty of Technology, University of Banja Luka, Bosnia and Herzegovina; the Faculty of Metallurgy, University of Zagreb, Sisak, Croatia; the Faculty of Natural Sciences and Engineering, University of Ljubljana, Slovenia; and the Faculty of Metallurgy and technology, University of Podgorica, Montenegro.

Financial support from the Ministry of Science, Technological Development and Innovation of the Republic of Serbia to researchers from Serbia for attending the congress is gratefully acknowledged. The support of the sponsors and their willingness to cooperate have been of great importance for the success of MME SEE 2023. The Organizing Committee would like to extend their appreciation and gratitude to all sponsors and friends of the conference for their donations and support.

We would like to thank all the authors who have contributed to this book of abstracts and also the members of the scientific and organizing committees, reviewers, speakers, chairpersons, and all the conference participants for their support of MME SEE 2023. Sincere thanks to all the people who have contributed to the successful organization of MME SEE 2023.

On behalf of the 5th MME SEE Scientific and Organizing Committee

Miroslav Sokić, PhD

PREPARATION AND CHARACTERIZATION OF CHITOSAN–CLAY COMPOSITES AS POTENTIAL DRUG CARRIERS

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In this study, the preparation and characterization of chitosan–clay composites as potential carriers for tetracycline were performed. The composites based on bentonite (from the deposit of Beretnica, Republic of Serbia) and chitosan were prepared. Two types of chitosan were used for the preparation of the composites: commercial low molecular weight chitosan and chitosan isolated from fruit bodies of the commercially grown mushroom *Agaricus bisporus*. Adsorption of tetracycline hydrochloride onto natural clay and chitosan-clay composites from aqueous media (pH 3.4) was studied. The amounts of adsorbed antibiotic were determined spectrophotometrically from the difference between the initial concentrations of tetracycline and the amounts remaining in the aqueous medium after dynamic equilibrium. Characterization of the natural clay and chitosan-clay composites before and after adsorption of tetracycline was performed by zeta potential measurements.

Adsorption of tetracycline followed a nonlinear isotherm for the natural clay and composites. The Langmuir equation showed the best fit of the equilibrium adsorption data over the entire concentration range (0.05 – 1 mg/mL) and the maximum adsorbed amount of the drug on the natural clay and both chitosan-clay composites was up to ~ 200 mg/g. Only minor differences in the adsorption of the drug were observed between the composites containing commercial chitosan and the chitosan isolated from mushrooms. The observed decrease in zeta potentials of the drug chitosan–clay complexes revealed the interactions of the composite surface with tetracycline molecules. The obtained results indicate the potential of the chitosan-clay composites for the development of therapeutic systems for topical application and suggest the possibility of using fungi as a renewable source of chitosan.

Keywords: clay, chitosan, composites, drug carriers, tetracycline

Introduction

Modern pharmacy and medicine impose the need to find advanced therapeutic systems, which will be more rational, efficient, and eco-friendly at the same time. Traditionally, a variety of minerals have been used as excipients in pharmaceutical dosage forms due to certain desirable physical and physicochemical properties, such as high adsorption capacity, high specific surface area, good swelling ability, thixotropy, plasticity, opacity, and color. The discovery that natural clays can act as drugs or coadjuvants in pharmaceutical preparations has greatly expanded the scope of their application (García-Villén et al. 2019). To improve the adsorption of organic molecules, functionalization with cationic substances has been used for modification of natural clays, enhancing their properties for drug adsorption (Khatoon et al. 2020).

Chitosan, a natural cationic polysaccharide, has attracted considerable attention as a functional, renewable, non-toxic, and biodegradable biopolymer for various pharmaceutical/biomedical applications (Zhao et al. 2018). It is well known that chitosan composites are used in medical practice (e.g., burn treatment, tendon or blood vessel engineering), but also in biosensors, packaging, separation processes, food or agricultural industries, and catalytic processes (Fatullayeva et al. 2022). Of the particular interest is possibility of using modified chitosan-clay composites as carriers for substances with antimicrobial activity (Ambrogi et al. 2017; Čalija et al. 2020; Huang et al. 2019). The tetracyclines are bacteriostatic antibacterials with a broad spectrum of activity.

Although topical application carries the risk of sensitisation and may contribute to the development of resistance, tetracycline hydrochloride has been used in pharmaceutical preparations for topical application (Buckingham 2020).

In this study composites based on bentonite and chitosan were prepared and the adsorption of tetracycline hydrochloride by the obtained composites was further investigated. Drug adsorption by the prepared composites was investigated by determination of the adsorption isotherms and zeta potential measurements with respect to the possible use of these materials as potential carriers for antibiotics.

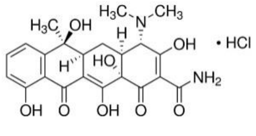
Materials and methods

A Ca-bentonite from the Beretnica deposit in the Republic of Serbia (BB) was used as the starting material. Two types of chitosan were used for clay modification, *i.e.*, preparation of the composites: commercial low molecular weight chitosan (Sigma-Aldrich, USA) (LCS) and chitosan isolated from fruit bodies of the commercially grown mushroom *Agaricus bisporus* (Delta Danube d.o.o., Kovin, the Republic of Serbia) (CSA). In brief, chitosan solution (25 mg of chitosan in 50 mL of 1% acetic acid solution) was gradually added to 100 mL of bentonite suspension (10% m/m) at a temperature of 60 °C. The suspension was stirred at a speed of 6000 rpm for 30 min. Prepared composites were filtered, air dried overnight, and ground to achieve particles below 63 μm. Composites are designated as BLCS and BCSA.

The physicochemical properties of the model drug are listed in Table 1. Adsorption of tetracycline hydrochloride (Sigma-Aldrich, USA) (TCH) on the mineral starting material and the prepared composites was carried out in batch experiments at room temperature. Aqueous stock solutions of the drug (pH 3.4) with different initial concentrations (0.05 – 1 mg/mL) were prepared in distilled water. Batch experiments were performed by shaking the mixture containing 40 mg of each composite and 10 mL of each drug solution on a laboratory shaker at 250 rpm for 1 h at room temperature and then separating after centrifugation at 3000 rpm for 30 min. Supernatants were used to determine the non-adsorbed drug concentrations. The initial and non-adsorbed drug concentrations were determined spectrophotometrically at λ=276 nm (Evolution 300 spectrophotometer, Thermo Fisher Scientific, UK). The amounts of adsorbed antibiotic were calculated from the difference between the initial concentrations of tetracycline and the amounts remaining in the aqueous medium after dynamic equilibrium.

Characterization of the natural clay and both chitosan-clay composites before and after adsorption of tetracycline was performed by zeta potential measurements of 0.1 mg/mL composite dispersions in deionized water using a Zetasizer NanoZS90 instrument (Malvern Instruments, UK).

Table 1 Structural formula and physicochemical properties of the model drug (PubChem n.d.)

Active pharmaceutical ingredient	Structural formula	Molecular Weight (MWt)	pKa
Tetracycline hydrochloride *(4S,4aS,5aS,6S,12aS)-4-(Dimethylamino)-3,6,10,12,12apentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12aoctahydrotetracene-2-carboxamide hydrochloride*		480.9	pKa (strongest acidic) 3.26 pKa (strongest basic) 9.25

Antibacterial activity of chitosan and clay samples were tested against one Gram-positive bacteria, *Enterococcus faecalis* (ATCC 29219) and one Gram-negative bacteria *Escherichia coli* (ATCC 25922). Broth microdilution assay was used for chitosan activity test and Plate-count method were applied for examination of clay (leachate and suspension) activity test.

Results and discussion

The adsorption of the drug by the natural clay and the chitosan-clay composites (BLCS and BCSA) was studied by determining the adsorption isotherms. The isotherms were obtained by plotting amounts of the drug adsorbed per unit weight of each adsorbent (mg/g) against the equilibrium concentrations of drug in solution (mg/L) and are shown in Figure 1.

It is observed that for the natural clay as well as chitosan-clay composites, adsorption of TCH increased with increasing of its initial concentrations. For the three adsorbents, adsorption of TCH followed a nonlinear adsorption isotherm and experimental data were analyzed by Langmuir and Freundlich models. The Langmuir equation showed better fit of the equilibrium adsorption data over the entire concentration range and the calculated maximum adsorbed amounts of TCH were: 256.1 mg/g for the natural clay, 232.6 mg/g for chitosan-clay composite BLCS and 263.2 mg/g for chitosan-clay composite BCSA. Slightly lower adsorption of TCH was achieved by BLCS, and only minor differences in the adsorption of the drug were observed between the natural clay and the chitosan isolated from mushrooms. Results showed that the natural clay and both chitosan-clay composites have high affinities for adsorption of TCH. The high adsorption of TCH by the natural clay indicates existence of the active sites in the interlayer space and at the external surface of clay relevant for adsorption of the investigated drug. The fact that presence of chitosan in chitosan-clay composites did not significantly influence the adsorption of tetracycline suggests that either the active sites in the natural clay were not covered by modification of clay with chitosan or in chitosan-clay composites, new active sites were formed at which TCH can be adsorbed. Also, since the highest adsorption of TCH was achieved by chitosan-clay composite obtained by modification of the natural clay with chitosan isolated from mushrooms points that this material may be especially interesting for the potential practical application as carrier for TCH.

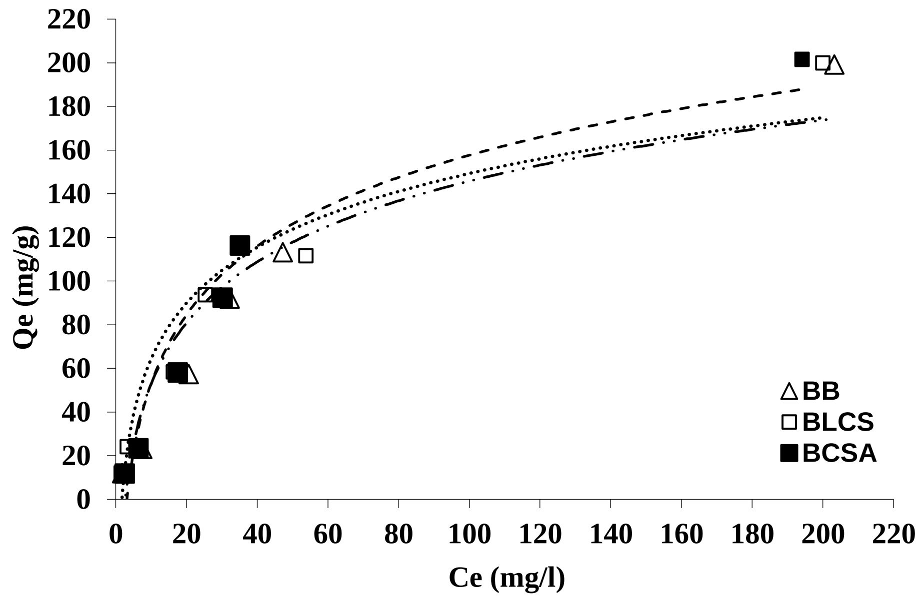


Figure 1 Adsorption of TCH by natural clay (BB) and chitosan-clay composites (BLCS and BCSA)

To confirm the interactions of TCH with the active sites at the surface and/or in the interlayer space of the natural clay and chitosan-clay composites, zeta potential, which is reflection of the surface charge of minerals, was determined. The results of the zeta potential measurements are shown in Figure 2. The negative zeta potential value (-29.3 mV) of the natural clay confirmed its negative surface charge and is characteristic for bentonite.

It was noticed that after modification of the natural clay with LCS, zeta potential become less negative (-26.1 mV), while for chitosan-clay composite containing CSA, zeta potential was practically unchanged (-29.7 mV). After adsorption of TCH, the following zeta potential values were obtained: -35.9 mV for the drug-the natural-clay complex - BB-TCH and -46.0 mV for BLCS-TCH and -34.2 mV for BCSA-TCH for the drug-chitosan-clay complexes. Results suggested interactions between TCH and active sites in the natural clay and chitosan-clay composites.

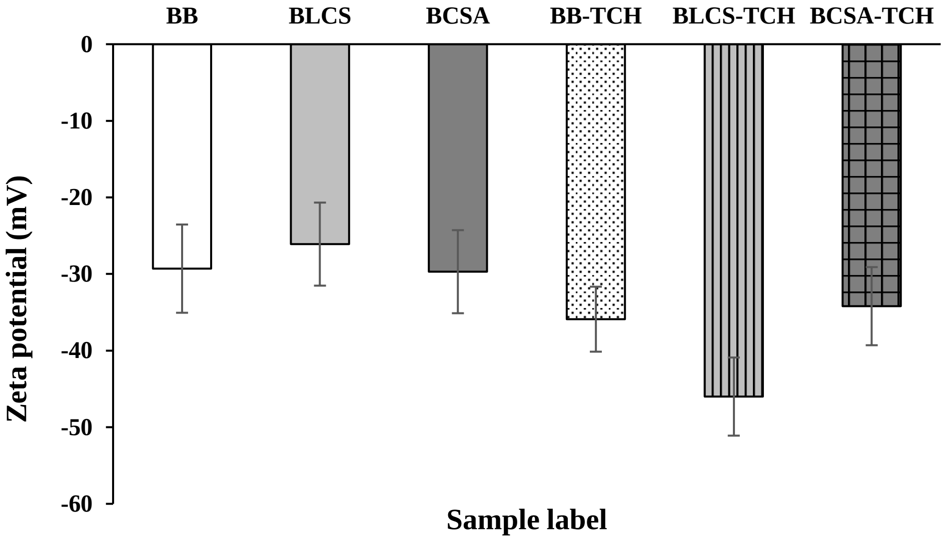


Figure 2 Zeta potentials of: natural clay (BB), chitosan-clay composites (BLCS and BCSA) and composites after drug adsorption (BB-TCH, BLCS-TCH and BCSA-TCH)

In addition, it has been found that by altering the zeta potential of mineral carriers, optimal binding and subsequent release of the active ingredient can be achieved (Kumar et al. 2020).

In the preliminary testing of antimicrobial activity, the commercial chitosan showed bactericidal concentration against *E. faecalis* at concentration of 1.25 mg/mL and 0.625 mg/mL against *E. coli*, while the chitosan isolated from *Agaricus bisporus* showed bactericidal activity at concentrations of 0.625 mg/mL against *E. faecalis* and 2.5 mg/mL against *E. coli*. The results of the antibacterial activity of the clay samples prepared as leachate and suspension against one Gram-positive and one Gram-negative bacteria are shown in Table 2. It was found that the number of bacterial colonies was not significantly reduced (less than 1 log) compared to the positive control samples.

Table 2 Antibacterial activity of clays leachate and suspension expressed as $\log_{10}$ (CFU/mL)

Clay sample	***Escherichia coli***	***Enterococcus faecalis***
Control	9.26 ± 0.22	9.02 ± 0.02
BB leachate	9.22 ± 0.07	8.24 ± 0.06
BB suspension	9.15 ± 0.15	9.30 ± 0.30

Considering that the obtained results indicate the lack of antimicrobial activity of the starting clay and the confirmed antimicrobial activity of both chitosans, it is expected that the prepared drug-clay composites with tetracycline will have improved antimicrobial properties. Based on the obtained results, a more detailed study of the antimicrobial spectrum of the obtained composites and investigation of the potential synergistic effect between chitosan and the drug will be performed.

Conclusion

The results obtained indicate the potential of the tested newly synthesized chitosan-clay composites for the development of drugs for topical application on infected and damaged skin. The study also pointed out an extremely important aspect, which is the possibility of using fungi as a sustainable source of chitosan.

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References

Ambrogi, Valeria, Donatella Pietrella, Morena Nocchetti, Serena Casagrande, Veronica Moretti, Stefania De Marco, and Maurizio Ricci. "Montmorillonite–Chitosan–Chlorhexidine Composite Films with Antibiofilm Activity and Improved Cytotoxicity for Wound Dressing." *Journal of Colloid and Interface Science* 491 (April 1, 2017): 265–72. https://doi.org/10.1016/j.jcis.2016.12.058.

Buckingham, Robert. 2020. *Martindale: The Complete Drug Reference*. London, Uk: Pharmaceutical Press.

Čalija, Bojan, Jovana V. Milić, Nikola Milašinović, Aleksandra Daković, Kata Trifković, Jovica Stojanović, and Danina Krajišnik. "Functionality of Chitosan-halloysite Nanocomposite Films for Sustained Delivery of Antibiotics: The Effect of Chitosan Molar Mass." *Journal of Applied Polymer Science* 137, no. 8 (February 20, 2020): 48406. https://doi.org/10.1002/app.48406.

Fatullayeva, Sevda, Dilgam Babir Tagiyev, N.A. Zeynalov, Samira Mammadova, and E. Aliyeva. "Recent Advances of Chitosan-Based Polymers in Biomedical Applications and Environmental Protection." *Journal of Polymer Research* 29, no. 7 (June 6, 2022). https://doi.org/10.1007/s10965-022-03121-3.

García-Villén, Fátima, E. Carazo, Ana Borrego-Sánchez, Rita Sánchez-Espejo, Pilar Cerezo, César Viseras, and Carola Aguzzi. "Clay Minerals in Drug Delivery Systems." In *Elsevier EBooks*, 129–66. Elsevier BV, 2019. https://doi.org/10.1016/b978-0-12-814617-0.00010-4.

Huang, Yan-Ping, Nianhua Dan, Weihua Dan, and Weifeng Zhao. "Reinforcement of Polycaprolactone/Chitosan with Nanoclay and Controlled Release of Curcumin for Wound Dressing." *ACS Omega* 4, no. 27 (December 19, 2019): 22292–301. https://doi.org/10.1021/acsomega.9b02217.

Khatoon, Nafeesa, Mao Xiang Chu, and Chun Hui Zhou. "Nanoclay-Based Drug Delivery Systems and Their Therapeutic Potentials." *Journal of Materials Chemistry B* 8, no. 33 (August 26, 2020): 7335–51. https://doi.org/10.1039/d0tb01031f.

Kumar, Ashok, Benjamin K. Hodnett, Sarah P. Hudson, and Peter Davern. "Modification of the Zeta Potential of Montmorillonite to Achieve High Active Pharmaceutical Ingredient Nanoparticle Loading and Stabilization with Optimum Dissolution Properties." *Colloids and Surfaces B: Biointerfaces* 193 (September 1, 2020): 111120. https://doi.org/10.1016/j.colsurfb.2020.111120.

PubChem. "PubChem." PubChem, n.d. https://pubchem.ncbi.nlm.nih.gov/.

Zhao, Dongying, Shuang Yu, Beini Sun, Shuang Gao, Sihan Guo, and Kai Zhao. "Biomedical Applications of Chitosan and Its Derivative Nanoparticles." *Polymers* 10, no. 4 (April 23, 2018): 462. https://doi.org/10.3390/polym10040462.