# XXVII Conference of Polish Chitin Society

# „NEW ASPECTS ON CHEMISTRY AND APPLICATION OF CHITIN AND ITS DERIVATIVES"

**September 21-23rd 2022**

**Polish Chitin Society**

**XXVII Conference**

**„New aspects on chemistry and application of chitin and its derivatives"**

**Scientific Committee:** Prof. Małgorzata JAWORSKA, Ph.D., D.Sc. – Honorary President of PTChit
Katarzyna STRUSZCZYK-ŚWITA, Ph.D. – President of PTChit

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**The conference is under the honorary patronage of prof. dr. hab. eng. Teofil Jesionowski, Rector of the Poznań University of Technology.**

POZNAN UNIVERSITY OF TECHNOLOGY

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# SCHEDULE OF CONFERENCE

## September 21st 2022 – Wednesday

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| **$16^{00}$-$19^{00}$** | **Registration** |
| **$19^{00}$-$22^{00}$** | **Buffet dinner** |

## September 22nd 2022 – Thursday

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| **$9^{00}$-$9^{30}$** | **Opening ceremony**<br>**Katarzyna Struszczyk-Świta, Ph.D.** |
| **$9^{30}$-$9^{55}$** | **Prof. Henryk Struszczyk prize - giving ceremony** |

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| **$9^{55}$-$10^{20}$** | | **Opening lecture**<br>**Prof. Samuel Hudson, Ph.D.**<br>**COMMENTS ON THE CHEMICAL CHARACTERIZATION OF CHITOSAN AND THE DEACTIVATION OF CHEMICAL CONTAMINANTS SUCH AS ENDOTOXIN** |
| **Session A** | | **Biological session** |
| **Chairman** | | **Prof. Małgorzata Jaworska, Ph.D., D.Sc** |
| **A1** | **$10^{20}$-$10^{40}$** | **M. Lootsik, N. Manko, M. Lutsyk (Jr.), R. Stoika**<br>**IMPROVED CHARACTERIZATION OF MOLECULAR WEIGHT PROFILES OF CHITOSAN SPECIMENS BY ELECTROPHORESIS IN POLYACRYLAMIDE GEL OF STEP GRADIENT POROSITY** |
| **A2** | **$10^{40}$-$11^{00}$** | **Tomasz Machałowski, Hermann Ehrlich, Teofil Jesionowski**<br>**DIVERSE VIEWS IN NATURALLY FORMED 3D CHITINOUS SCAFFOLDS AND THEIR PRACTICAL UTILITY** |
| **$11^{00}$-$11^{25}$** | | **Coffee/tea break** |

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| **Session B** | | **Medical session** |
| **Chairman** | | **Radosław Wach, Ph.D.** |
| **B1** | **$11^{25}$-$11^{45}$** | **Katarzyna Kresse-Walczak, Heike Meissner, René Mauer, Evelyn Trips, Klaus Boening**<br>**VALIDATION OF A NOVEL ARTIFICAL BIOFILM EQUIVALENT FOR DENTURES – A PROSPECTIVE PILOT STUDY** |
| **B2** | **$11^{45}$-$12^{05}$** | **Dorota Chełminiak-Dudkiewicz, Aleksander Smolarkiewicz-Wyczachowski, Kinga Mylkie, Paweł Nowak, Katarzyna Węgrzynowska-Drzymalska , Dariusz T. Młynarczyk, Marta Ziegler-Borowska**<br>**CHITOSAN-BASED FILMS INCORPORATED WITH NATURAL ACTIVE SUBSTANCE AS A POTENTIAL WOUND DRESSING** |
| **B3** | **$12^{05}$-$12^{25}$** | **Vladyslav Vivcharenko, Michał Wójcik, Krzysztof Pałka, Agata Przekora**<br>**EFFECTS OF THE PRODUCTION METHODS ON THE PROPERTIES OF CHITOSAN/AGAROSE BIOMATERIAL** |
| **B4** | **$12^{25}$-$12^{45}$** | **Joanna Potaś, Agnieszka Wilczewska, Paweł Misiak, Anna Basa, Katarzyna Winnicka**<br>**OPTIMIZATION OF MULTILAYER FILMS COMPOSED OF CHITOSAN AND LOW METHOXY AMIDATED PECTIN AS MATERIALS FOR BUCCAL DRUG DELIVERY** |
| **$13^{00}$-$14^{00}$** | | **Lunch** |
| **$14^{30}$-$19^{00}$** | | **Visiting the monuments of Poznań with a guide**<br>***Ostrów Tumski - Old Town - Imperial Castle*** |
| **$20^{00}$-$24^{00}$** | | **Gala dinner** |

**September 23rd 2021 – Friday**

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| **$9^{00}$-$10^{00}$** | | **General Assemble of the Polish Chitin Society**<br>*(only for PTChit members)* |
| **Session C** | | **Physico-chemical session** |
| **Chairman** | | **Katarzyna Małolepsza-Jarmołowska, Ph.D., D.Sc.** |
| **C1** | **$10^{00}$-$10^{20}$** | **Irina Kuznik, Iris Kruppke, Chokri Cherif**<br>**PROCESS DEVELOPMENT OF A WET-SPINNING PROCEDURE FOR PURE CHITOSAN FILAMENT YARNS USING IONIC LIQUIDS** |
| **C2** | **$10^{20}$-$10^{40}$** | **Dominik Sikorski, Marta Bauer, Justyna Frączyk, Beata Kolesińska, Dorota Kręgiel, Zbigniew Draczyński**<br>**PRELIMINARY STUDIES ON THE PREPARATION, MODIFICATION, AND MODULATION OF THE PROPERTIES OF CHITOSAN MATERIALS FOR MEDICAL USE** |
| **$10^{40}$-$11^{00}$** | | **Coffee/tea break** |

| Session D | Poster session $11^{00}$-$12^{30}$ |
|---|---|
| P1 | Katarzyna Lewandowska, Marta Szulc<br>FILM-FORMING PROPERTIES OF COMPOSITE FILMS BASED ON CHITOSAN, CLAY AND LACTIC ACID |
| P2 | Katarzyna Lewandowska, Natalia Jermakow, Maria Doligalska<br>NEMATODE INFECTION INDUCES DEPOSITION OF CHITIN-LIKE STRUCTURES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE |
| P3 | Małgorzata Gnus<br>SPINEL FERRITES – ORIENTATION IN CHITOSAN MEMBRANES AND THEIR EFFECT ON WATER AND ETHANOL TRANSPORT PROPERTIES IN THE PERVAPORATION PROCESS |
| P4 | Urszula Filipkowska, Tomasz Jóźwiak, Konrad Karczmarczyk<br>THE USE OF CHITIN FROM MEALWORM MOLTS MILLER (TENEBRIO MOLITOR) FOR THE REMOVAL OF CATIONIC DYES FROM AQUEOUS SOLUTIONS |
| P5 | Tomasz Jóźwiak, Urszula Filipkowska, Beata Bralewska<br>THE USE OF CHITIN FROM THE MOULTS OF THE MEALWORM (TENEBRIO MOLITOR) FOR THE REMOVAL OF ANIONIC DYES FROM AQUEOUS SOLUTIONS |
| P6 | Agnieszka Dzieniszewska, Janusz Nowicki, Grzegorz Rzepa, Justyna Czupioł, Izabela Semeniuk, Damian Kiełkiewicz<br>CHITOSAN MODIFIED WITH IMIDAZOLIUM IONIC LIQUID AS AN ADSORBENT FOR REMOVAL OF PHOSPHATE FROM AQUEOUS SOLUTIONS |
| P7 | Yara Gaber Martel, Heike Meißner, Klaus Böning, Katarzyna Kresse-Walczak<br>EFFECTS OF CHITOSAN-BASED DENTURE CLEANSER ON DENTAL MATERIALS |
| P8 | Kinga Mylkie, Paweł Nowak, Marta Ziegler-Borowska<br>SYNTHESIS OF CHITOSAN WITH FREE DIHYDROXYBORYL GROUPS FOR MONOSACCHARIDE BINDING |
| P9 | Magdalena Gierszewska, Ewa Olewnik-Kruszkowska<br>DIALDEHYDE STARCH DERIVATIVES AS A CROSSLINKERS OF BIOPOLYMERS |

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| **P10** | **Zofia Nuc, Gloria Brusotti, Paola Moro, Laura Catenacci, Chiara Milanese, Milena Sorrenti, Aldona Dobrzycka-Krahel, Cristina Bonferoni, Carla Caramella**<br>**ASTACUS LEPTODACTYLUS (ESCHSCHOLTZ, 1823) IN CIRCULAR ECONOMY** |
| **P11** | **Anna Michalicha, Agata Przekora , Dawid Stefaniuk, Magdalena Jaszek, Anna Matuszewska, Anna Belcarz**<br>**SELECTED OXIDOREDUCTASES IMMOBILIZED VIA A POLYDOPAMINE LAYER INCREASE BIOLOGICAL SAFETY OF CURDLAN** |
| **P12** | **Bożena Grimling, Bożena Karolewicz**<br>**EFFECT OF CHITOSAN ADDITION ON PHYSICOCHEMICAL PROPERTIES OF DERMATOLOGICAL APPLICATIONS CONTAINING HUMULUS LUPULUS L. EXTRACT** |
| **P13** | **Katarzyna Małolepsza-Jarmołowska**<br>**CHITOSAN GELS TO PREVENT REFLUX** |
| **P14** | **Katarzyna Małolepsza-Jarmołowska**<br>**RESEARCH ON INCREASING THE EFFECTIVENESS OF GELS WITH CHITOSAN PROTECTING THE ESOPHAGEAL MUCOSA** |
| **P15** | **Marcin Wysokowski, Krzysztof Nowacki, Michał Niemczak, Maciej Galiński, Teofil Jesionowski**<br>**IONIC LIQUID-ASSISTED SYNTHESIS CHITIN-ETHYLENE GLYCOL HYDROGELS AS ELECTROLYTE MEMBRANES FOR SUSTAINABLE ELECTROCHEMICAL CAPACITORS** |
| **P16** | **Klaudia Piekarska, Maria Wiśniewska-Wrona, Olga Marchut-Mikołajczyk, Katarzyna Struszczyk-Świta, Piotr Drożdżyński**<br>**PREPARATION AND CHARACTERIZATION OF A BIOCOMPOSITE OF CHITOSAN AND GLYCOLIPIDS FOR AGRICULTURAL APPLICATIONS** |
| **P17** | **Katarzyna Struszczyk-Świta**<br>**MICROWAVE-ASSISTED EXTRACTION OF CHITOSAN AND LIPIDS FROM THE BIOMASS OF FILAMENTOUS FUNGI** |
| **P18** | **Izabela Dziedzic**<br>**ISOLATION OF PORIFERAN CHITIN SCAFFOLDS USING METHANOL WITH ASSISTANCE OF ULTRASOUND TREATMENT** |
| **$12^{30}$-$12^{50}$** | **Closing of the conference** |
| **$13^{00}$-$14^{00}$** | **Lunch** |

**Szymon Mania**
*Gdańsk University of Technology, Faculty of Chemistry, Department of Chemistry, Technology and Biotechnology of Food, G.Narutowicza 11/12 Str., Gdańsk Poland*

## IS THERE ANY PLACE FOR CHITOSAN IN 3D PRINTING TECHNOLOGY?

Today, 3D printing in the world is primarily method of rapid prototyping. The new applications of the available additive manufacturing techniques act on the imagination and new possibilities, but still represent a small percentage of the entire income pool in this industry. High hopes related to 3D printing are placed on the possibility of using it in the biomedical and food production sectors, as those most related to the quality of human life. A device that works in three dimensions is not enough for this purpose. Therefore, scientists are very interested in materials that can be layered in a controlled manner and are human-friendly, such as natural polymers including chitosan. The presentation shows what looked like the beginning of 3D printing, why it has become so popular in recent years, and how previous scientific works have allowed to develope the biomedical and food industries. How many percent of chitosan can be contained in a thermoplastic matrix for printing by fused deposition modeling? [1,2]. Can the sol-gel transformation of chitosan solution be so fast that it can be used to create 3D objects? [3]. Will we be able to produce food adapted to our diet or personalized dressings adapted to the wound with based on chitosan scaffolds with incorporated living cells? These are just a few pressing questions about the future of chitosan in 3D printing.

*Acknowledgements:*

*Financial support of there studies form Gdańsk University of Technology by the DEC-10/2021/IDUB/I.3.3. grant uder the AGENTUM 2: Design a multihead 3D printing system for production of biocompatibile scaffolds based on the unstable form of chitosan hydrogel doped with agarose. - 'Excellence initiative - Research University' program is gratefully acknowledgement.*

References:

[1] Mania S., Ryl J., Jinn J-R., Wang Y-J., Michałwoska A.,Tylingo R., The production possibility of the antimicrobial filaments by co-extrusion of

the PLA pellet with chitosan powder for FDM 3D printing technology, Polymers (2019) 1893.

[2] Tylingo R., Kempa P., Banach-Kopeć A., Mania S., A novel method of creating thermoplastic chitosan blends to produce cell scaffolds by FDM additive manufacturing, Carbohydrate Polymers (2021) 110028.

[3] Banach-Kopeć A., Mania S., Tylingo R. Badania kompozytu chitozan-agaroza jako potencjalnego akładnika biotuszu do druku 3D. (2022) Implanty conference. Gdańsk, 27-29.05.2022.

# *OPENING LECTURE*

**S. Hudson**

*Chief Scientific Officer at Karamedica, Inc., Raleigh, North Carolina, US*

## COMMENTS ON THE CHEMICAL CHARACTERIZATION OF CHITOSAN AND THE DEACTIVATION OF CHEMICAL CONTAMINANTS SUCH AS ENDOTOXIN

It is essential to well characterize chitosan chemically, in order that chemical experiments may be replicated. Since chitosan is derived from natural products, such as crustacea shells, by batch processes, it is difficult to obtain chitosan that is exactly replicated, from batch to batch. Critical parameters characterizing chitosan include the degree of deacetylation, the molecular weight, the molecular weight distribution, and the distribution of remaining acetyl groups. Depending on how the chitosan is obtained, this distribution may tend towards a blocky arrangement or could be random. Additionally, if chitosan is to be employed for internal medical applications, endotoxins must be removed or deactivated down to approximated 2ng/g of chitosan. Recent results using an atmospheric plasma process on chitosan to remove endotoxins will be discussed.

# *Session A*

# *BIOLOGICALL SESSION*

**M. Lootsik [1], N. Manko [1], M. Lutsyk (Jr.) [2], R. Stoika[1,2]**

[1] - *Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv*

[2] - *Danylo Halytsky Lviv National Medical University, Lviv, Ukraine*

## IMPROVED CHARACTERIZATION OF MOLECULAR WEIGHT PROFILES OF CHITOSAN SPECIMENS BY ELECTROPHORESIS IN POLYACRYLAMIDE GEL OF STEP GRADIENT POROSITY

**Background**. Chitosan is a biocompatible and biodegradable natural biopolymer that is widely used in different fields of biology, medicine, pharmacy and food industry. However, its biological effects significantly depend on the physico-chemical characteristics, mainly upon the degree of polymerization (DP) and degree of deacetylation (DDA). These and other indicators strongly affect the viscosity and water solubility of chitosan – properties which are very important for its scientific and commercial application. It should be stressed that the values of above listed parameters of chitosan specimens presented in the catalogues of different companies do not reflect the real degree of heterogeneity of chitosan molecules. In order to solve the problem of chitosan diversity, a costly method of high performance-size exclusion chromatography (HP-SEC) was proposed, however, it is not convenient for use by the chitosan specialists.

**The aim** of our study was to develop a convenient procedure for determination of the molecular weight profile of chitosan specimens by means of electrophoresis in the step gradient porosity of polyacrylamide gel.

**Results**. Electrophoresis in polyacrylamide gel of step gradient porosity (2.5, 3.5, 5.0, 10.0, 15.0, 20.0, 25.0 % w/v) was proposed for characterization of native chitosan and its molecular weight derivatives.

The pool of chitosan molecules in samples was separated in type 1 gel in up to 6 fractions. The main amount of chitosan was accumulated in the molecular weight region from 550 to 40 kDa and it was manifested as three electrophoretic bands which differed significantly by their ratio in different samples. Electrophoresis of chitosan fragments fractionated by column gel-permeation chromatography on Acrylex P-30 provided distinct separation of 3–40 kDa molecular weight fragments in gel.

It was found that chitosan fragments of different molecular weight were characterized by different hemostatic, cytotoxic (towards human tumor cells) and anti-fungal effects.

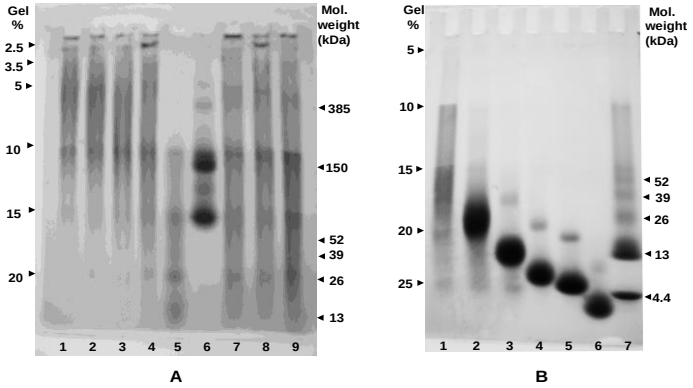


**Figure**. Electrophoretic pattern of native chitosan specimens and its fragments in PAAG with step-gradient porosity due to PAA concentration in gel.

Section A: "Chitopharm" L (1), "Chitopharm" M (2), "Chitopharm" S (3), chitosan "Aldrich" (4), cytochrome C oligomers (5), human Igs (6), chitosan "Organica" (7), chitosan "BioProgress 300", native chitosan (8), and chitosan after treatment with 0.5 M HCl in 0.5 M acetic acid for 1 h at 80° C (9).

Section B: low molecular fragments of honeybee chitosan after its hydrolysis and gel filtration on Acrylex P-30 column. 1. honeybee chitosan (initial sample), 2-6 – fractions of chitosane hydrolysate eluted from Acrylex P-30 column, 7 – molecular weight markers (clupein - 4.4 kDa, cytochrom C (13 kDa) and its oligomers.

**Conclusion**. Regiment of electrophoresis of chitosan in step-gradient porosity of polyacrylamide gel was developed. It permits characterization of the molecular weight profile of polymer chains in chitosan specimens and showed its effectiveness in monitoring chitosan fragments in range of molecular weights from 3 to 400 kDa isolated by gel permeation chromatography.

**Publications**

1. Lootsik M.D., Bilyy R.A., Lutsyk M.M., Stoika R.S. Preparation of chitosan with high blood clotting activity and its hemostatic potential assessment. ***Biotechnologia Acta***. 2015, Vol. 8, N6. P.32-40. doi 10.15407/biotech8.06.032.

2. Lootsik M. D., Bilyy R. O., Lutsyk M. M., Manko N. O., Navytka S. A., Kutsiaba V. I., Stoika R. S. Honeybee (*Apis mellifera*) chitosan: purification, heterogeneity and hemocoagulating activity. ***Biotechnologia Acta***. 2016. Vol. 9, N6. P. 39-49. https://doi.org/10.15407/biotech9.06.039.

3. Lootsik M., Manko N., Gromyko O., Tistechok S., Lutsyk M.(Jr.), Stoika R. Honeybee chitosan-melanin complex: isolation and investigation of antimicrobial activity / ***Ukrainian Biochemical Journal***. 2020. Vol. 92, N 6, P.143-153. doi: https://doi.org/10.15407/ubj92.06.143.

**Tomasz Machałowski** [1], **Hermann Ehrlich** [2,3], **Teofil Jesionowski** [1]

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[2] – *Institute of Electronic and Sensor Materials, TU Bergakademie Freiberg, Freiberg, Germany*

[3] – *Center for Advanced Technology, Adam Mickiewicz University, Poznan, Poland*

## DIVERSE VIEWS IN NATURALLY FORMED 3D CHITINOUS SCAFFOLDS AND THEIR PRACTICAL UTILITY

Recently, biological materials isolated from renewable sources such as plants, microorganisms, or animals remain to be very interesting propositions of scaffolds. Their strong similarity to the extracellular matrix (ECM), minimize chronic inflammation response, commonly recorded during synthetic polymers application [1]. Chitin is widely distributed structural polysaccharide in the nature, second most abundant in the world after cellulose [1]. Its extraordinary features as well-developed chemical structure, biocompatibility, biodegradability, and renewability makes it interesting candidate for several modern application [3]. Nevertheless, limitations resulting from structural properties of chitin such as difficult solubility significantly limit its practical utility. Commercial chitin that is traditionally isolated from fungal biomass (i.e., *Aspergillus niger, Mucor rouxii*) or crustaceans shells (crabs, lobsters, shrimps, crayfish, king crabs) allow to obtain form of granules, sheets or powder — not as 2D or 3D scaffolds what additionally exacerbates these problems.

The solution may be to look for an alternatives for the synthetically produced scaffolds and to obtain them in the right quantity directly from the source [302]. Therefore, the purpose of this study was the determination of structural and physicochemical properties of naturally prefabricated 3D chitin.

The unique, hierarchical three-dimensional spatial structure and mechanical properties of chitin scaffolds isolated from marine sponges predispose these bioconstructions to various applications in tissue engineering, regenerative medicine, and environmental protection study. Herein, we present the observations of the cells culture using natural chitinous skeletons isolated from *Aplysina fistularis* marine sponge. The experiments have been carried out with human dermal keratinocytes (HaCaT), human fibroblasts (NHDF), murine fibroblasts (Balb / 3T3), and human osteoblasts cells (hFOB 1.19, ATCC® CRL-11372™). Several

biophysical parameters have been determined, including, cytotoxicity of the scaffold, surface roughness or mechanical properties. Further functionalization of the scaffold by covering bio$CaCO_3$ particles allow to improve osteoblasts cells spreading and viability. Then, utility of the biosystem composed of chitin-nano$SiO_2$- horseradish peroxidase( HRP) for removal contaminations of synthetic 17a-ethinylestradiol (EE2) from the aqueous solutions has been determined. In this case, HRP was immobilized onto nanosilica powder (by adsorption and trapping) and on chitinous scaffold isolated from the *Aplysina fistularis* marine sponge (by adsorption). Impact of the several process parameters onto EE2 removal efficiency has been determined including temperature, pH, concentration of EE2 or amount of $H_2O_2$. Our diverse views on naturally formed chitinous skeletons shown their versatility and high potential for future applications.

Acknowledgements:
This work was partially supported by the Polish Ministry of Education and Science. Additionally, OPUS 19 project of National Science Centre, Poland with the grant number 2020/37/B/ST5/01909.

References:
[1] D.K. Singh, A.R. Ray, Biomedical applications of chitin, chitosan, and their derivatives, J. Macromol. Sci. Part C Polym. Rev. 40 (2000) 69–83.
[2] T.-L. Yang, Chitin-based materials in tissue engineering: Applications in soft tissue and epithelial organ, Int. J. Mol. Sci. 12 (2011) 1936-1963
[3] V. V. Mutsenko, V. V. Bazhenov, O. Rogulska, D.N. Tarusin, K. Schütz, S. Brüggemeier, et al., 3D chitinous scaffolds derived from cultivated marine demosponge *Aplysina aerophoba* for tissue engineering approaches based on human mesenchymal stromal cells, Int. J. Biol. Macromol. 104 (2017) 1966–1974.

# *Session B*

# *MEDICAL SESSION*

**Katarzyna Kresse-Walczak [1], Heike Meissner [1], René Mauer [2], Evelyn Trips [3], Klaus Boening [1]**

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*[2] – Carl Gustav Carus Faculty of Medicine, Technische Universität Dresden, Institute for Medical Informatics and Biometry (IMB), Fetscherstr. 74, Germany*

*[3] – Carl Gustav Carus Faculty of Medicine, Technische Universität Dresden, Coordination Centre for Clinical Trials, Fetscherstr. 74, Germany*

## VALIDATION OF A NOVEL ARTIFICIAL BIOFILM EQUIVALENT FOR DENTURES - A PROSPECTIVE PILOT STUDY

The correlation between oral biofilm and oral, and general diseases is well known [1]. Therefore, cleaning teeth and dentures is essential for the maintenance of oral and general health [2]. The use of suitable artificial biofilm equivalents may be helpful, to efficiently perform oral and denture hygiene education, and to carry out in vitro examinations of oral hygiene products. The aim of this pilot study was to verify the feasibility of the study protocol, design, and methods for the development of a validated artificial biofilm equivalent (ABE) for removal dentures.

The clinical part of this single group, prospective, longitudinal, interventional pilot study was conducted in Dresden (Germany) from March until December 2020. The medical ethical committee from TU Dresden concede the ethical approval No. EK108032019. The participants wore sufficient upper complete dentures. The recruitment occurred via flyers and posters. The main outcome measures were descriptions of the feasibility of the study protocol, design, methods, and reliability. Additionally, data about biofilm collection, biofilm removability by brushing, and ABE mixtures were recorded. Denture biofilm was collected for 12 h and 36 h on dentures (parts 1 and 2) and standardized acrylic specimens (parts 3 and 4) using intraoral appliances. The cylindrical specimens (Ø12.75 mm, height 1.5 ± 0.5 mm) were manufactured with polymethylmethacrylate (PMMA, Palapress clear, Kulzer, Hanau, Germany). The ABE containing chitosan (ChS, Chitosan 90/500, Chitoceuticals, molecular weight 200-400 kDa, degree of deacetylation 87.6%-92.5%, Heppe Medical Chitosan GmbH, Halle, Germany) and methylcellulose (MC, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was validated. The brushing strokes (BS) and percentage of plaque (POP) were measured. A computerized planimetric method (CPM) was used to

evaluate the remaining biofilm or ABE after brushing. Descriptive statistics was used to evaluate the data.

31 participants were pre-screened, 8 (26%) were included and allocated to intervention. One participant did not complete the intervention due to the termination of the study. Participants who refused (n = 4, 12%) gave no reasons or "no time" as a reason. All participants wore an upper denture and four of them also lower denture. The mean age of the participants was 77 ± 5 years. Recruitment strategy and retention have been stated as successful. No adverse events were reported. The intraoral appliances attahed to the dentures were well tolerated, and biofilm could be collected. The reliability of removal of ABE was indicated.

The study protocol, design, and methods were feasible and reliable for conducting the future main study. For the main study, a reduction of study parts and a focus on the specimens, as well as extended recruitment are worth consideration.

*Acknowledgements:*

*The study was suported by DFG Grant No: 427547277*

References:

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[2] Araujo CB, Ribeiro AB, Fortes CV, et al., Effect of local hygiene protocols on denture-related stomatitis, biofilm, microbial load, and odor: A randomized controlled trial, Journal of Prosthetic Dentistry, 20 (2021) S0022-3913.

**Dorota Chełminiak-Dudkiewicz [1], Aleksander Smolarkiewicz-Wyczachowski [1], Kinga Mylkie [1], Paweł Nowak [1], Katarzyna Węgrzynowska-Drzymalska [1] , Dariusz T. Młynarczyk [2], Marta Ziegler-Borowska [1]**

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## CHITOSAN-BASED FILMS INCORPORATED WITH NATURAL ACTIVE SUBSTANCE AS A POTENTIAL WOUND DRESSING

Skin wounds are one of the most common injuries worldwide. In recent years, accidental skin injuries have increased rapidly, leading to many patients with skin wound disease [1]. Although many materials have been developed to accelerate wound healing, obtaining a single dressing that meets all of these conditions remains a challenge. Currently, many of the dressings consist mainly of synthetic polymers. Natural polymers that show compatibility with the human body and biological processes seem to be a better option.

Chitosan is considered a preferred material due to its high molecular weight, excellent biocompatibility, and hemostatic properties, making it widely used in wound healing. In addition, chitosan is an inexpensive polymer, which is important for a potential dressing material that will be affordable for the patient. Furthermore, the properties of chitosan cause it to form so-called active dressings that control the biochemical state of the wound [2-3]. However, there is still a risk of infection. To prevent this, it is necessary to involve the active substances into such materials further to improve the properties of the bioactive wound dressing.

This study focuses on obtaining and characterizing a novel chitosan-based biomaterials containing cannabis oil for potentially promoting wound healing. The primary active substance in cannabis oil is the non-psychoactive cannabidiol, which has many beneficial properties. Three chitosan-based films containing different concentrations of cannabis oil were prepared. Along with increasing the amount of oil, the obtained biomaterials became rougher as detected by atomic force microscopy (AFM). Such a rough surface promotes protein adsorption, which was

confirmed by studying the interaction of human albumin with the obtained materials. Increasing the oil concentration also improved the films' mechanical parameters, swelling capacity, and hydrophilic properties, as examined by measuring the wetting angle. On the other hand, increasing the amount of oil decreased the water vapor permeability, essential in wound dressing. Furthermore, the prepared films were subjected to an acute toxicity test using a Microtox, and the results suggest their antimicrobial activity. Significantly, the film's increased cannabis oil content enhances the antimicrobial effect against A. fisheri for films in direct contact with bacteria.

*Acknowledgements:*

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## EFFECTS OF THE PRODUCTION METHODS ON THE PROPERTIES OF CHITOSAN/AGAROSE BIOMATERIAL

Progressive bioactive wound dressings are the main goal of the new approach to wound repair [1]. There is a great demand for new smart products that will not only cover the wound, but also support the wound regeneration process [2]. Therefore, it is essential to test various production methods in order to obtain the most promising wound dressing [3]. The aim of the research was to fabricate a biomaterial dedicated for skin wound healing based on two marine-derived polysaccharides (chitosan and agarose) using two different methods: freeze-drying and air-drying techniques. It should be noted that the composition of the biomaterials was identical, while the production method differed in the final step, where either air drying was used to obtain the chitosan agarose film or freeze drying to obrain the foam-like biomaterial. Due to the selected method of biomaterials production, they differed significantly in their properties, which consequently determined their potential application in regenerative medicine. The use of the air-drying method resulted in a thin biocompatible film that promoted cell growth on its surface and could be used as a potential artificial skin substitute. Its biodegradable nature can ensure its gradual replacement by the newly formed patient tissue [4,5]. Application of the freeze-drying method resulted in obtaining a highly porous and biocompatible foam-like material, which was not supportive to cell adhesion on its surface and could be potentially used as a highly absorbent external wound dressing. As a result, it will provide painless replacement, ensuring simple and comfortable changing of the dressing [6].

Despite the fundamental differences related to the cellular properties, the biomaterials also differed in their mechanical and physicochemical properties. Based on the conducted research, it can be concluded that the selection of an appropriate production method results in obtaining unique features of the biomaterial. Despite the same

composition of the samples, the deciding factor also belongs to the chosen drying method. To better understand the influence of drying on the obtained properties, additional tests comparing both samples (ATR FTIR, XPS) are required.

*Acknowledgements:*

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## OPTIMIZATION OF MULTILAYER FILMS COMPOSED OF CHITOSAN AND LOW METHOXY AMIDATED PECTIN AS MATERIALS FOR BUCCAL DRUG DELIVERY

Materials based on polyelectrolyte complexes (PECs) have been under detailed investigation over the last years as structures with great possibilities in tissue engineering and chemical or pharmaceutical technology. They are characterized by unique properties arising from combining polymers with different characteristics enriched with special properties of PECs structures [1].

Polyelectrolyte multilayers (PEMs) being consisted of alternately deposited layers of anionic and cationic polyions separated with ionically interacting chains of neighboring polymers – PECs structures – are recognized as particularly interesting materials dedicated for manufacturing either functionalized coatings or drug delivery platforms [1].

Among the plethora of polyelectrolytes being utilized for PEMs technology, systems with cationic chitosan (CS) have been widely investigating. Considering the increasing potential of PECs materials with CS as novel and multifunctional drug carriers and our previous experience in PECs – based systems fabrication, the idea was to dive deeper into technological aspects of interpolymer complexation process and verify whether selection of the internal and external stimuli might be helpful in developing the optimized CS/pectin (PC) materials for buccal drug delivery.

In this study, medium molecular weight CS and low methoxy amidated PC (LM PC) with different calcium reactivity were utilized for the films preparation. PC - as an anionic biopolymer with carboxylic acid groups capable for ionic interactions with positively charged CS - has been under careful investigation for PECs formation, for many years [2]. Layer – by – layer deposition of the polyelectrolyte solutions according to the solvent evaporation technique was aimed to eliminate the risk of PECs precipitation since the preliminary studies pointed out high susceptibility of CS/PC blends to the phase separation. In the framework of PEMs optimization, different PC type, polymer ratios and the order of polyelectrolyte mixing was applied. Besides the detailed mechanical

analysis with using Texture Analyzer TA.XT. Plus (Stable Microsystems, Godalming, UK), thermal properties of the films were evaluated by Mettler Toledo Star TGA/DSC unit (Columbus, OH, USA). The internal multilayer structure of the composites was picturized with scanning electron microscopy (SEM), although, the visual assessment was equally important since the films were morphologically diversified.

Analysis of the mechanical properties and visual observations of the CS/LM PC PEMs enabled to select the most promising composites with simultaneous elimination of those regarded as not applicable due to very low uniformity and weak mechanical strength. As SEM images picturized, complex structure of the systems - CS and LM PC layers divided by PECs structures - was obtained. CS/LM PC polycomplex was characterized by the improved physicomechanical and thermal stability, and the presence of interpolymer bonds was confirmed i.a. in the differential scanning calorimetry (DSC) assay. Either the preparation technique or the polymer ratio were crucial for PEMs performance. The stoichiometric/non - stoichiometric character of the polycomplex determined its physicomechanical behavior.

*Acknowledgements:*

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# *Session C*

# *PHYSICO-CHEMICAL SESSION*

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## PROCESS DEVELOPMENT OF A WET-SPINNING PROCEDURE FOR PURE CHITOSAN FILAMENT YARNS USING IONIC LIQUIDS

The biopolymer chitosan (CHS) made from renewable biomass is non-toxic, biodegradable, highly compatible for colonization by human cells and provides the best conditions for use in biomedical applications [1, 2]. The properties of CHS, especially its degradation rate, mainly depends on the degree of deacetylation (DD). The challenging production and wet-spinning of CHS filament yarns has currently major limitations. Basically CHS with DD 85 – 95 % and high molecular weight (MW) (mostly expensive and/or medical grade CHS) is used for the fiber spinning yet. By using ionic liquids as solvents in a sustainable wet spinning process, other qualities of CHS (for example lower-cost CHS, food grade or low MW CHS) with DD ≤ 85 % can be processed into CHS filament yarns [3].

The ionic liquid 1-ethyl-3-methylimidazolium acetate (EmimOAc) was suc-cessfully used for manufacturing of pure chitosan mono- and multifilament yarns by a lab and pilot scale wet-spinning process. Chitosan of different qualities with 60 - 85 % DD was used for the preparation of spinning dopes with solids content of 4 – 8 wt.%. Rheology tests were carried out for the characterization of the viscometric properties. EmimOAc was used as a solvent and deionized water as coagulation and washing medium. Scanning electron microscope (SEM), infrared spectroscopy (FT-IR) and textile physical tests were carried out for the evaluation of the structural and mechanical characteristics of the CHS fibers. The manufactured chitosan filaments show a homogeneous structure with a diameter of 40 - 50 µm and 20 - 30 dtex yarn count. The mechanical tests show tensile strengths ≥ 20 cN/tex at Young's modulus up to 15 GPa. The research deals also with textile-technological processability of CHS filament yarns, as weaving, knitting or braiding. As a result of this AiF research project, a sustainable process for CHS filament yarn spinning is available to produce CHS yarns with high performance and functional capabilities.

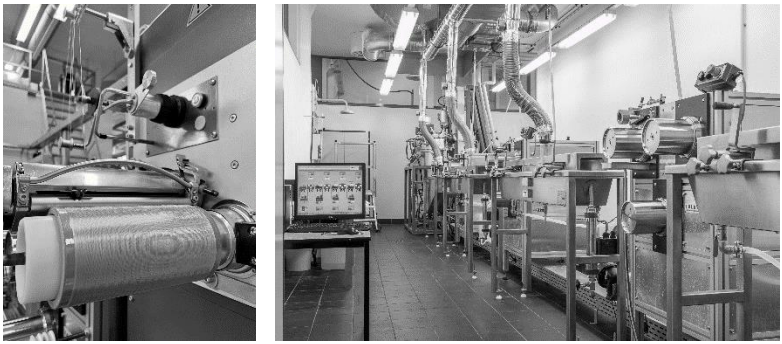

Figure 1: (left) Chitosan multifilament yarns spun with ionic liquids as a solvent; (right) Wet-spinning plant at the ITM

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# PRELIMINARY STUDIES ON THE PREPARATION, MODIFICATION, AND MODULATION OF THE PROPERTIES OF CHITOSAN MATERIALS FOR MEDICAL USE

Chitin is one of the most common polysaccharides in the natural environment. It can be found in the structure of sponges, corals, the shells of marine invertebrates, insects, and fungi cell walls[1-2]. It was isolated from mushrooms in 1811 by H. Braconnot, and its structure was described by A. Hofmann in 1930 [3]. Chitosan is a biopolymer, which is a derivative of chitin, widely available in various forms and used by various industries. The difference between chitin and chitosan is in the degree of deacetylation [4]. Chitosan is used as a starting material for the preparation of various forms such as membranes and sponges [5-6].

The antibacterial and antifungal properties of chitosan have been studied and described in many articles. The main antimicrobial activity of chitosan is due to electrostatic interactions between this cationic molecule and the negatively charged cell walls [7]. Additionally, the presence of two reactive groups (amine and hydroxyl) in chitosan opens opportunities for its chemical modification. These groups allow to carry out the sulfonation, amination, and carboxymethylation [8] reactions.

The aim of the work was to develop and carry out the physicochemical characterization of chitosan salts as derivatives of acetic, propionic, butyric, and valeric acids, as well as hydrochloric acid. The implementation of this goal can be used to obtain chitosan salts in the form of fibers without exposing them to the process of dissolving them in the water while forming the corresponding acid salt. As part of the research, it was also planned to perform pilot tests of the antibacterial activity of modified chitosan materials in order to select the most optimal chitosan

salts for further research on the use of various forms (nonwovens, films, spheres, and others) of modified chitosan. The antibacterial activity was planned to be determined against E. coli and S. aureus.

In addition, films were made from chitosans of different molecular weights and modified with classical antibacterial compounds. In this way, it was tested whether the molecular weight in the case of the films makes a difference in antibacterial activity.

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# *POSTER SESSION*

**P1**

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## FILM-FORMING PROPERTIES OF COMPOSITE FILMS BASED ON CHITOSAN, CLAY AND LACTIC ACID

Chitosan (Chit) materials based on composites and mixture are widely used in cosmetic, biomedical and packaging applications [1-4]. Additionally, their properties could be modified using different methods. Moreover, the type of solvent acid used a significant impacts the physicochemical properties of chitosan solutions and other materials, e.g. films or hydrogels [1,5].

In this study, the effect of clay type in chitosan composites on the rheological and physical properties of Chit solutions and films was investigated. The flow properties of Chit solution with and without clay were carried out using steady shear tests. The polymer films containing clay were prepared by solvent evaporation from the lactic acid solution. The properties of films were characterized by swelling behaviour and tensile tests. In addition, to modify properties of chitosan materials, such as mechanical properties and solubility of films, treatment with sodium hydroxide aqueous solution was used [6].

The obtained results were compared and showed that the addition of clay was favorable to the formation of intermolecular ineractions between chitosan and clay, which improved in the properties of these materials.

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**P2**

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## NEMATODE INFECTION INDUCES DEPOSITION OF CHITIN-LIKE STRUCTURES IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Studies aimed to identify the chitin-like structures in the brain of ageing C57Bl/6 mice infected with intestinal nematode, H. polygyrus. The experiments were conducted on male C57BL/6 mice and included uninfected control group (CTR), mice injected with bacteria endotoxin (LPS), mice injected with LPS and infected with H. polygyrus (LPS + Hp) and mice infected with H. polygyrus (Hp), only. To induce neuroinflammation [1-3], LPS solution was administered by intraperitoneal injection before nematode infection, at a total dose of 0.5625 mg per mouse. The mice were orally infected with 200 infective larvae (L3) of H. polygyrus. At the beginning of the experiment, animals were 16 weeks old and were examined at the age of 66 weeks. Selective staining was used to analyze the presence of chitinous-like structures in the slides of brain tissue. A Fluorescent Brightener 28 was used, which, when bound to the tissue, showed enhanced fluorescence. In the extracellular region, structures of different sizes and showing brightening of different intensities have been identified. The brightest structures were present in nematode-infected mice and particularly were enhanced in the LPS+HP group. No signal was detected in the tissues of control mice stained with calcofluoride. In light of the positive response to the presence of chitin-like polysaccharides in this study, infrared spectroscopy using attenuated total reflection (ATR) was used as a method to distinguish chitin-like substances in brain homogenate. The analysis confirmed the presence of chitin-like polysaccharides with spectra typical of chitin (Figure 1). Comparing the spectra of chitin (Chit) and N-acetylglucosamine (D-GlcNAc), a similar pattern of FTIR spectra were observed in all mice infected with H. polygyrus. FTIR analysis showed the presence of amide I at 1648 $cm^{-1}$ and the absorption bands of amide II and III in chitin samples were identified at 1542 $cm^{-1}$ and 1307 $cm^{-1}$. The bands of amide I, amide II and amide III indicated that the resulting three-

dimensional chitin is in the form of alpha crystallization. This is the first observation of chitin-like deposits in the brain of H. polygyrus-infected mice. In ageing mice with prolonged H. polygyrus infection, chitin-like deposits may reveal the induction of a new pathological mechanism in a mouse model of Alzheimer's disease.

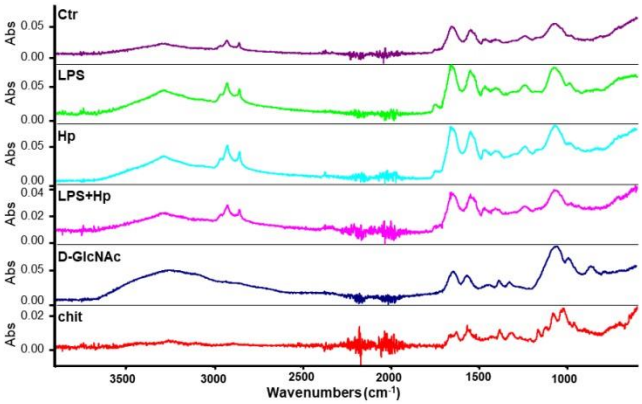


Figure 1. ATR-infrared spectra of various groups: Ctr - uninfected control group, LPS - mice injected with LPS, Hp - mice infected with H. polygyrus, LPS+HP - mice injected with LPS and infected with H. polygyrus, D-GlcNAc - N-acetylglucosamine, chit - chitin.

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**P3**

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## SPINEL FERRITES – ORIENTATION IN CHITOSAN MEMBRANES AND THEIR EFFECT ON WATER AND ETHANOL TRANSPORT PROPERTIES IN THE PERVAPORATION PROCESS

Ferromagnetic materials which are mainly composed of ferric oxide (α-$Fe_2O_3$) are called "ferrites". Depending upon their crystal structures ferrites can be categorized into three categories: spinel, garnet, and hexa-ferrites. Spinel ferrites are marked via the nominal formula MFe2O4, where M represents the divalent cations with an ionic radius ranging from 0.6 to 1 Å i.e. copper, magnesium, cobalt, nickel, zinc, etc. elements.

Magnetite, $Fe_3O_4$, which is a natural mineral, is a genuine ferrite, and its magnetism had been recognized in ancient times. However, the first attempt to prepare various types of ferrites and industrialize ferrites was not made until the beginning of the XX century. After that scientists find out unique physicochemical properties including excellent magnetic characteristics, high specific surface area, active surface sites, high chemical stability, tunable shape and size, and the ease with which they can be modified or functionalized. The exceptional properties of spinel ferrites make them promising materials for applications in various fields such as adsorbents [1,2], sensors [3,4], magnetic devices [5,6], photocatalysis [7], medicine [8,9], and membranes[10-12].

The aim of this work was to determine the influence of the content and distribution of spinel ferrites on chitosan membrane transport properties. For this purpose, a series of chitosan membranes containing different amounts of $Fe_3O_4$, $NiFe_2O_4$, $MnFe_2O_4$, and $MgFe_2O_4$ were prepared. Due to their magnetic properties, particles were arranged in the membrane matrix by an external magnetic field during membrane preparation. Transport properties of membranes prepared with and without magnetic field were tested in pervaporative ethanol dehydration process and compared.

The results of the research on the separation of the ethanol-water mixture showed that the presence of ferrites in the polymer matrix affects membrane transport properties and it depends on the distribution of nanoparticles.

Acknowledgements:

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**P4**

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## THE USE OF CHITIN FROM MEALWORM MOLTS MILLER (TENEBRIO MOLITOR) FOR THE REMOVAL OF CATIONIC DYES FROM AQUEOUS SOLUTIONS

The work examined the efficiency of sorption of cationic dyes Basic Violet 10 (BV10) and Basic Red 46 (BR46) popular in the industry on chitin obtained from mealworm molts miller (Tenebrio molitor) (ChMM). The scope of work included research on the effect of pH on the effectiveness of dye sorption, research on the kinetics of dye sorption (describing data with pseudo-first-order, pseudo-second-order models and intramolecular diffusion model) as well as determining the maximum sorption capacity of ChMM (describing experimental data with Langmuir 1 and 2 and Freundlich models).The efficiency of BV10 and BR46 sorption on ChMM was highest at pH 6. The sorption equilibrium time of the dyes on the tested sorbent ranged from 180 to 240 minutes and depended on the initial concentration of the dye. Data from the sorption kinetics of BV10 and BR46 on ChMM were best described by the pseudo-second-order model. Analysis of the intramolecular diffusion model indicated that the sorption of the tested dyes on the ChMM occurs in 3 main phases, differing in intensity and duration. The maximum sorption capacity of ChMM relative to BV10 and BR46 was 3.22 mg/g and 59.56 mg/g, respectively.

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**P5**

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## THE USE OF CHITIN FROM THE MOULTS OF THE MEALWORM (TENEBRIO MOLITOR) FOR THE REMOVAL OF ANIONIC DYES FROM AQUEOUS SOLUTIONS

In this study, the sorption efficiency of the industrial anionic dyes Reactive Black 5 (RB5) and Reactive Yellow 84 (RY84) on chitin from the moults of the mealworm (CMM) was investigated. The scope of this study included: effect of pH on sorption efficiency of dyes, determination of pHPZC of the tested sorbent, sorption kinetics of RB5 and RY84 on CMM (determination of sorption equilibrium time, description of data by pseudo-first- and pseudo-second-order models and by intramolecular diffusion model) and determination of maximum sorption capacity of CMM towards dyes (description of data by Langmuir I and II models and Freundlich model).The sorption of RB5 and RY84 on CMM occurred most efficiently at pH 3 and pH 2. The equilibrium time of sorption of RB5 and RY84 on CMM was 300 and 270 min, respectively. The experimental data of dye sorption kinetics were best described by a pseudo-second-order model. The analysis of the intramolecular diffusion model constants showed that the sorption of dyes on CMM occurs in 3 different time and intensity phases. The maximum CMM sorption capacities for RB5 and RY84 dyes were 121.15 mg/g and 138.55 mg/g, respectively, which were higher than most activated carbon-based materials (literature data). The sorption capacity of CMM was similar to that of chitin from shrimp carapaces (literature data) which suggests that insect moults are as good a source of chitin as marine crustacean carapaces.

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**P6**

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## CHITOSAN MODIFIED WITH IMIDAZOLIUM IONIC LIQUID AS AN ADSORBENT FOR REMOVAL OF PHOSPHATE FROM AQUEOUS SOLUTIONS

Pollutants entering the water environment contribute to its degradation and disruption of naturally occurring processes. The widespread use of phosphorus-containing products, including fertilizers or detergents, contribute to excess phosphorus in surface waters. Phosphorus is one of the essential elements necessary for the metabolism of living organisms, playing a key role in the functioning of ecosystems. Still, the large amounts of phosphate in the environment cause undesirable phenomena, i.e., leads to eutrophication of water bodies and thus the deterioration of water quality [1]. Adsorption is an attractive and widely used technique of phosphate removal because of its high efficiency, low price and simplicity [2]. Among many investigated materials, chitosan has gained much interest as a high-performance adsorbent due to its unique properties and ease of modification [3,4].

In this study, chitosan modified with glycidyl imidazolium ionic liquid (1-(2,3-epoxypropyl)-3-methylimidazolium chloride) was prepared and investigated for phosphate removal from aqueous solutions. The adsorption experiments before and after modification were conducted by a batch method as a function of pH (4-9), adsorbent dosage (1-6 g/L), initial phosphate concentration (1-200 mg/L) and contact time (2.5-360 min). The effect of the form of chitosan (powder, beads) on the adsorption process was also examined. The experimental data were fitted with various isotherm models (Freundlich, Langmuir, Dubinin-Radushkevich, Sips) and kinetic models (pseudo-first order, pseudo-second order, intraparticle diffusion model).

The results showed that the modification with ionic liquid had a significant effect on the adsorption capacity of chitosan. The adsorption process also depended on the form of chitosan and experimental conditions. Based on pH and adsorbent dosage studies, the optimal

conditions were determined as pH=5 and a dosage of 2 g/L. The maximum adsorption capacities of modified chitosan beads and powder were more than 7 and 3 times higher, respectively, compared to unmodified chitosan. At low initial concentrations (1-25 mg/L), the uptake of phosphate was higher by modified chitosan powder than beads, while as the initial concentration increased, chitosan beads adsorbed higher amounts of ions. The experimental data fitted best with the Sips isotherm, which indicates that the adsorption mechanism is complex and does not follow ideal monolayer adsorption. Kinetic studies showed that the adsorption on modified chitosan powder occurred immediately, while on chitosan beads the process was slower, and the system reached equilibrium after more than 3 hours. Kinetic models indicated that the process involves chemisorption and intraparticle diffusion. Phosphate adsorption on chitosan modified with imidazolium ionic liquid is attributed to the electrostatic attraction of negatively charged ions with positively charged surface of chitosan and the imidazolium cation.

Ionic liquid-modified chitosan has proved to be an effective adsorbent for phosphate removal.

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**P7**

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## EFFECTS OF CHITOSAN-BASED DENTURE CLEANSER ON DENTAL MATERIALS

Many conventional denture cleansers contain strong chemical agents that may be an environmental hazard and show adverse effects if used improperly. To avoid these risks, our intention was to develop a biocompatible and environmentally friendly denture cleanser. Due to their antimicrobial and anti-biofilm properties, chitosan, citric acid or acetic acid might be a good alternative to clean dentures.

Nevertheless, the effect of chitosan diluted in acetic acid or citric acid on denture materials is unknown. The aim of this in vitro study was to test different dental materials in terms of the effects of different chitosan acid mixtures on surface roughness.

A total of 120 cylindrical test specimens: polymethyl methacrylate (PMMA), polyethylene terephthalate (PET), cobalt-chromium molybdenum alloy (CoCrMo), polyamide 12 (PA12) with dimensions of 13.0 mm x 3.0 mm were prepared (n=30 each material). Roughness measurements were determined before and after immersion in artificial saliva (control group), 4% chitosan acetic acid (ChS-A), and 4% chitosan citric acid (ChS-C) (n=10 per group).

The chitosan solutions were prepared as follows: To obtain chitosan acetate mixture 480 mg acetic acid (Apotheke des Universitätsklinikums C.G. Carus) was mixed with 20 mg chitosan (Chitosan 90/100/A1, BioLog Heppe® GmbH, Landsberg, Germany). The mixture was placed on a magnetic stirrer at 150 rpm and 40°C and stirred for 12 h. To obtain chitosan citrate mixture 100 mg of citric acid monohydrate (VWR International GmbH, Darmstadt, Germany) was mixed with 400 mg distilled water and mixed on a magnetic stirrer for 15 min at a temperature of 60°C. After the powder has completely dissolved, 480 mg of this mixture was added to 20 mg of chitosan (Chitosan 90/100/A1, BioLog Heppe® GmbH, Landsberg, Germany). The mixture was stirred on a magnetic stirrer for 12 h with 150 rpm and a temperature of 40°C.

The test specimens were soaked in the artificial saliva and chitosan mixtures for a period of 14 days under dark, dry conditions, and room temperature to simulate 5 years cleaning period. Surface roughness was measured using a profilometer (Hommel Etamic W20 (Fa) Jenoptik Industrial Metrology Germany GmbH, Villingen, Schweningen) at 6 points per specimen.

The statistical analysis was performed using IBM SPSS Statistics software version 28.0.1.0. To identify normal distribution the Kolmogorov-Smirnov, the Shapiro- Wilk Test, and histograms were used. The Kruskal-Wallis test and U-test for pairwise group comparison were used. The $\alpha = 0.05$ was determined. P-values were adjusted with the Bonferroni-Holm-Method.

The specimens were divided into 12 groups (n = 10 each). Within each dental material, the modified groups were compared with the control groups.

## Results

The roughness parameters (Ra) after immersion in the media were not normally distributed. The Kruskal-Wallis-Test showed a p-value of $< 0.001$. After the adjustment the p-values did not show statistically significant differences in any groups. The lowest Ra-value was found in PMMA soaked in chitosan acetic acid (Ra = 0.03 µm) and the highest Ra-value in polyamide 12 group soaked in chitosan citric acid (0.17 µm). All values did not overpass the clinical acceptable value of 0.2 µm for surface roughness. All Ra values before and after soaking in the respective media showed only minor changes in roughness.

## Conclusion

High surface roughness leads to faster accumulation of microorganisms and ultimately to the formation of biofilm on intraoral surfaces. This can have a negative impact on oral health, but also on the esthetics and durability of the dental restoration. The result of this study indicates that a chitosan-based denture cleanser can be used to clean removable dentures and does not interfere significantly with the surface roughness of the tested dental materials.

**P8**

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## SYNTHESIS OF CHITOSAN WITH FREE DIHYDROXYBORYL GROUPS FOR MONOSACCHARIDE BINDING

Chitosan is a polysaccharide derived from chitin; it is biocompatible, biodegradable, and non-toxic; therefore it can be used in medicine as an antibacterial biomateriał [1]. Functional groups and O-glycosidic bonds present in the chitosan structure (hydroxyl, amine) show very high chemical activity. This allows for their modification by e.g. introducing other functional groups. This can improve the chemical and physical properties of this polysaccharide as well as broaden its uses [2].

The main goal of the project was to modify chitosan, which would have highly reactive functional groups able to bind quickly saccharides present in glycoproteins.

The first stage of the project involved the chemical modification of chitosan to produce dialdehyde chitosan and then introducing into its structure dihydroxyboryl groups derived from boronic acids capable of fast sugar-binding. The obtained new polymer material was characterized in terms of structure and surface morphology (ATR-FTIR, SEM, TEM, and XRD analyzes). Employing thermogravimetric analysis and differential scanning calorimetry, the thermal stability of the material, important from the point of use, was determined. The hydrophilic/hydrophobic nature of the modified chitosan was also investigated by measuring the contact angle. The characterized material was then tested for the ability to bind monosaccharides, such as glucose. The amount of monosaccharide bound was determined by a chemical method. The chemical method used the reducing properties of sugars and consisted of the reduction of 3,5-dinitrosalicylic acid (DNS) in an alkaline environment and at high temperatures to a colored product that could be determined spectrophotometrically. The results obtained in this way allowed the assessment of the material's ability to quickly bind glycosidic units contained in glycoproteins.

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**P9**

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## DIALDEHYDE STARCH DERIVATIVES AS A CROSSLINKERS OF BIOPOLYMERS

After World War II the packaging industry was revolutionized by the introduction of synthetic polymers. Plastics have nearly dominated the packaging market in the 2nd half of the 20th century, rapidly increasing their volumetric contribution to the waste stream. Recently raising concern over environmental pollution casts a new light on polymer packaging. The growing demand for eco-friendly and biodegradable packaging materials became a driving force for new inventions in packaging chemistry [1]. Naturally occurring polysaccharides, e.g. chitosan, proteins, and their derivatives have been widely proposed as substitutes for synthetic materials, particularly for the manufacturing of packaging films.

Many researchers have obtained chitosan-based films, adding also other functional materials into it to produce composite films with enhanced, e.g. preservative, properties. As neat chitosan films do not present reasonable physical properties from the package industry point of view like tensile strength and elasticity, many efforts are needed to develop the chitosan-based films to meet the practical application criteria and can successfully compete with the petroleum-based packaging films. The main problem arises from the solubility of Ch-based films in contact with water and aqueous media, in many cases main components of food.

The aim of this study assumes the possible crosslinking of the Ch with natural starch derivative, dialdehyde starch (DAS), to prevent Ch films swelling and solubility in an aqueous environment. The chemical structure, surface morphology, mechanical properties of Ch/DAS films was evaluated. Special attention was devoted to the swelling properties in different external fluids (water, biological buffers, simulated food fluids).

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**P10**

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## ASTACUS LEPTODACTYLUS (ESCHSCHOLTZ, 1823) IN CIRCULAR ECONOMY

Narrow-clawed crayfish(Astacus leptodactylus, Eschscholtz, 1823) is a native species to Europe and Western Asia [1]. According to FAO[2], it ranks fifth in terms of production of crayfish species in global aquaculture. As much as crayfish nutritious meat is used as food, shells remain as a waste. It is important to include these wastes into a circular economy, for example, as a source of chitin. Moreover, since the standard, industrial method used to obtain this valuable polymer is not so environmentally friendly, it is important to work on methods with the minimal impact on the environment[3]. One of them is using natural deep eutectic solvents mixtures (NADES), which are a mixture of two or more primary metabolites. They are considered safe for the environment and non-toxic. Furthermore they can be reused [4]. In this study we used the modified method described by Zhu et al., 2017 [5] for chitin extraction from narrow-clawed crayfish shells, to see if it is possible to obtain chitin in the greener way, and if it is suitable for industrial use. To perform the extraction, first, the material was treated with 10% citric acid (10ml/1g)at the room temperature. Then the NADES mixture was prepared (choline chloride : malonic acid in molar ratio 1:2) at 100°C, and mixed with material in ratio 10:1. Dried material was characterized with FTIR, TGA, and SEM. The final product presented the amide I bands at 1650 $cm^{-1}$ 1620 $cm^{-1}$ and the amide II band at 1550 $cm^{-1}$ confirming that the samples corresponded to standard chitin. The yield of chitin constituted 29% of the dry carapax weight. Stability studies confirmed that the obtained chitin is similar to the respective standard samples. Therefore, it is possible to obtain a good quality chitin from narrow-clawed crayfish using more sustainable reagents than highly concentrated HCl and NaOH. Currently the studies are in progress to further deacetylate the chitin using a green process to obtain chitosan. This research was carried out as part of an Erasmus + international exchange programme shared between the University of Gdansk and the University of Pavia.

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**P11**

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## SELECTED OXIDOREDUCTASES IMMOBILIZED VIA A POLYDOPAMINE LAYER INCREASE BIOLOGICAL SAFETY OF CURDLAN

The need to provide dressing materials with new additional functions prompted us to functionalize the curdlan hydrogel in order to increase its suitability for this purpose. Unfortunately, curdlan is a biopolymer that is not susceptible to modifications, while most of chemical modifications destroy curdlan's structure. Additionally, curdlan does not have active groups in its structure that can bind biologically active molecules. Our previous results showed that curdlan hydrogel modified with polylevodopamine (poly(L-DOPA)) layer can be a promising platform for the production of functional dressing materials. The functional polycatecholamine layer enabled the functionalization of hydrogel with gentamicin molecules [1].

Therefore, we decided to verify whether such a coating would allow the modification of curdlan using oxidoreductase enzymes (laccase and peroxidase). The functionalization of the curdlan hydrogel was performed by post-setting polydopamine or poly(L-DOPA) deposition, using copper ions as a process catalyst in some variants. Poly(L-DOPA) coating was found to be more efficient in terms of immobilized enzymes yield, and these matrices also showed notable enzymatic activity.

The modified matrices and the control curdlan sample resulted in impaired metabolism of human dermal fibroblasts (HDFs) compared to the negative control for this assay. However, a cell viability test showed that all biomaterials were non-toxic. The non-toxicity of the tested samples was confirmed by the experiment of direct contact between the tested materials and human fibroblasts. All of the manufactured modifications increased the biomedical safety of the curdlan hydrogel by inhibiting the cytokines synthesis by human macrophages. The tested matrices in contact

with blood did not significantly affected blood-clotting ability, but induced partial blood haemolysis.

Summing up, despite the lack of toxicity and the ability to inhibit the macrophages response, the produced matrices are controversial candidates for dressing materials due to unfavorable parameters in contact with blood. However, they could be considered for the production of dressing materials for the treatment of non-bleeding wounds.

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**P12**

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## EFFECT OF CHITOSAN ADDITION ON PHYSICOCHEMICAL PROPERTIES OF DERMATOLOGICAL APPLICATIONS CONTAINING HUMULUS LUPULUS L. EXTRACT

The aim of this study was to obtain the optimal qualitative and quantitative composition of a dermatological application for use in the treatment of inflammatory skin conditions. For this purpose, hop extract obtained from Humulus Lupulus L was introduced by supercritical CO2 extraction as a therapeutic substance into selected gels base. The obtained applications were subjected to tests of physicochemical properties and pharmaceutical availability for the extract main component of cohumolone isolated from semi-solid dosage form with the most characterized favorable parameters. The gels were subjected to the following pharmaceutical tests: visual evaluation of homogeneity, spreading, rheological, texturometric, consistency, blurring and content of the active substance[1-2].

For the study, Celugel (Hydroxyethylcellulosi mucilago), which is a hydrophilic base in the treatment of skin conditions such as exudative lesions, burns, acne, other diseases associated with oily skin, for use also on hairy skin and mucous membranes, was used. Celugel, combined with hops cone extract, takes the form of a liquid gel, leaving a fast-drying membrane on the skin with cooling properties. The addition of 2% chitosan results in a thickened and less watery consistency of the base [3].

Studies demonstrated that the presence of chitosan in the Celugel-based carrier prevents the precipitation of large agglomerates of hop extract in the formulation. Chitosan thickens the structure of the hydrophilic base, binding twice as much extract in the gel improving its application and rheological properties compared to Celugel without the addition of chitosan. Pharmaceutical availability studies of cohumulone from Celugel vehicle with the addition of 2% chitosan showed a decrease in the average percentage of released active ingredient of 13.81% cohumulone at a constant release rate of 0.004610 h-1 compared to the application formulation with Celugel base without chitosan. The tested

formulations provide a great range of possibilities for matching properties to the disorder and skin requirements.

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**P13**

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## CHITOSAN GELS TO PREVENT REFLUX

Gastroesophageal reflux is the term used to describe the reverse flow of acid gastric contents into the esophagus. The problem of acidic reflux is still not effectively resolved, as evidenced by articles in the available literature. Among the recommended drugs are causative agents that reduce the amount of secreted acid and preparations to alleviate the symptoms of the disease. The obtained results of experimental studies show the possibility of obtaining preparations preventing damage to the esophageal mucosa as a result of gastroesophageal reflux [1-3].

The aim on the work was to investigate the influence sodium alginate on the properties of chitosan-containing gels.

All investigation gels contained chitosan. The effect of chitosan on the properties of gels was investigated. The formulations were prepared with various pH and rheological properties. The tests gels shows the adhesion work. The pH range of the gels allows the selection of the optimal preparation. On the basis of the tests, the dynamic viscosity of gels was determined. The obtained results of experimental studies have shown that it is possible to produce a preparation with optimal pharmaceutical and application properties. Gels show the adhesion and the ability to cover the surface of the apparatus simulating the conditions in the esophagus. Due to their adhesive properties, the tested gels should stay on the esophageal mucosa for a long time and protect it against the adverse effects of gastric or bile contents. The wide range of pH of the investigated gels enables selection of a preparation with optimal pH for the esophagus. Additionally, gels with alkaline pH could neutralize acids. Presented assumptions and investigations in vitro require verification in vivo, what is the aim of subsequent studies.

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## RESEARCH ON INCREASING THE EFFECTIVENESS OF GELS WITH CHITOSAN PROTECTING THE ESOPHAGEAL MUCOSA

Current recommendations for patients with GERD are based on alleviating the symptoms of the disease. Hydrogels are designed to protect the esophageal mucosa against harmful factors. An important aspect of the protective effect of gels is their sufficiently long time to adhere to the mucosa. The conducted research indicates the possibility of obtaining preparations helping to solve this problem [1-3].

The aim of the study was to investigate the influence of poloxamer 407 on the adhesive properties of gels containing chitosan.

The thermosensitive polymer, poloxamer 407, used in the research has many advantageous features that are useful in achieving the research goal. This polymer has the ability to increase viscosity. Its presence in the gels may increase the adhesion of the preparation. Its addition increases the pH of the gels. The formulations were prepared with various pH and rheological properties. All investigation gels contained chitosan. The effect of chitosan on the properties of gels was investigated. On the basis of the tests, the dynamic viscosity of gels was determined. Gels show the adhesion and the ability to cover the surface of the apparatus simulating the conditions in the esophagus. On the basis of performed investigations in vitro, it may be assumed that the gels will remain at the site of application in the form of a layer coating the mucous membrane of the oesophagus and protecting it against an irritating effect of gastric content backflow. A wide range of pH of the investigated gels enables selection of a preparation with optimal pH for the esophagus. Additionally, gels with alkaline pH could neutralize acids. The poloxamer 407 added to the gels allow for the differentiation of pH depending on the type of reflux. The investigated gels, thanks to their adhesive properties, should remain on the mucous membrane of the oesophagus for a prolonged time and protect it

against the unfavourable effect of gastric content. Laboratory tests require clinical confirmation.

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**P15**

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## IONIC LIQUID-ASSISTED SYNTHESIS OF CHITIN-ETHYLENE GLYCOL HYDROGELS AS ELECTROLYTE MEMBRANES FOR SUSTAINABLE ELECTROCHEMICAL CAPACITORS

A novel chitin–ethylene glycol hybrid gel was prepared as a hydrogel electrolyte for electrical double-layer capacitors (EDLCs) using 1-butyl-3-methylimidazolium acetate [Bmim][Ac] as a chitin solvent. Examination of the morphology and topography of the chitin–EG membrane showed a homogeneous and smooth surface, while the thickness of the membrane obtained was 27 µm. The electrochemical performance of the chitin–EG hydrogel electrolyte was investigated by cyclic voltammetry and galvanostatic charge/discharge measurements. The specific capacitance value of the EDLC with chitin–EG hydrogel electrolyte was found to be 109 F g−1 in a potential range from 0 to 0.8 V. The tested hydrogel material was electrochemically stable and did not decompose even after 10,000 GCD cycles. Additionally, the EDLC test cell with chitin–EG hydrogel as electrolyte exhibited superior capacitance retention after 10,000 charge/discharge cycles compared with a commercial glass fiber membrane.

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## PREPARATION AND CHARACTERIZATION OF A BIOCOMPOSITE OF CHITOSAN AND GLYCOLIPIDS FOR AGRICULTURAL APPLICATIONS

Currently, preparations used in agriculture for plant protection and/or plant growth stimulation are mainly compounds obtained in the process of chemical synthesis [1]. Unfortunately, these products are not neutral to the environment and human health. The global trend of searching for new alternatives to synthetic chemical compounds has also reached agriculture, forcing researchers to develop alternative plant protection products based on natural resources. Biopolymers are widely tested [2]. Among them, chitosan deserves special attention, because it is successfully used in agriculture due to its non-toxicity, antimicrobial activity and induction of protective reactions in plant tissues. In addition, chitosan is a non-toxic and biodegradable polymer [3-5].

The presented research is aimed at the production of an advanced biocomposite containing chitosan with glycolipids - biosurfactants, which are the metabolic products of *Bacillus pumilus* 2A endophytic bacteria. The prepared biocomposite containing two bioactive molecules of natural origin was tested to stimulate plant growth. So far, it has been proven that biosurfactants, surface-active molecules, biodegradable and non-toxic, can stimulate plant growth and protect against pathogenic microorganisms [6].

Biocomposites were prepared containing chitosan in concentrations of 0.98-1.00% (w/v) and glycolipids in amounts of 5 and 15% (w/v). The parameters of the obtained biocomposites, such as pH value and dynamic viscosity, as well as changes in chitosan structure, were characterized by FTIR spectroscopy. Their effect on the stimulation of seed germination was evaluated based on Phytotoxkit tests (MicroBioTests Inc.). In the procedure, inhibition, presence and growth of seeds were measured after 72 hours of exposure to biopolymeric compounds in the soil. In the

control samples, seeds were hydrated with water. The Phytotoxkit tests were carried out using seeds of *Sorghum saccharatum* and *Lepidium sativum*.

In the course of the research, effective and environmentally friendly preparations to be used in agriculture have been prepared [7]. The results of our research showed that biocomposites of chitosan and glycolipids increased the growth of the tested plants, both roots and stems. The most promising results were observed for cress watered with the chitosan lactate with the addition of 5% (w/v) of glycolipids where the length of the stalk was 100% higher than in samples hydrated with water and the length of the root was 80% higher compared to the control sample.

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## MICROWAVE-ASSISTED EXTRACTION OF CHITOSAN AND LIPIDS FROM THE BIOMASS OF FILAMENTOUS FUNGI

Since the mid-1980s, microwave radiation has been accepted in chemistry as an efficient method of heating reaction mixtures. Chemical processes that require several hours of conventional heating, thanks to the use of microwaves can be carried out in just a few minutes, additionally they run with high efficiency and selectivity. Due to their environmental friendliness, low byproduct production, ease of use, and quickness, microwave assisted reactions are significant and quickly developing processes in the so-called green chemistry (often automated). Microwave energy is used in chemically and enzymatically catalysed reactions [1]. Additionally, the isolation of a variety of substances from microbial cells, such as lipids or biopolimers, is enhanced by the use of microwaves [2-3].

The aim of the research was to develop a method of isolating lipids and chitosan from the cells of *Mucor circinelloides* IBT-83 filamentous fungi with the use of microwave energy. A specialized CEM Discover SP-D microwave reactor was used in the tests.

It was shown that treating biomass with microwave energy of 300 W power for 20 minutes in a mixture of organic solvents hexane-isopropanol (3:2, v/v), (process temp. 30°C, pressure 2500 Pa) is an effective method of lipid extraction from mold cells. Under these conditions, it was possible to obtain 34.05 ± 0.6% of lipids. After lipid extraction, the residual biomass was once more microwaved in an alkaline environment (200 W, 30 min, 30°C, 2500 Pa) to separate chitosan. The use of microwave energy allowed for a 10% increase in the amount of extracted lipids compared to the conventional method (not assisted by microwaves). In comparison to the yield of 6.51% ± 0.5% (w/w) of dry biomass for the conventional extraction method, a higher yield of 12.10 ± 0.6% (w/w) of chitosan from fungal biomass was obtained.

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## ISOLATION OF PORIFERAN CHITIN SCAFFOLDS USING METHANOL WITH ASSISTANCE OF ULTRASOUND TREATMENT

Poriferan chitin skeletons are characterised by the unique 3D architecture that has been shown to allow diverse application such as scaffolds for tissue engineering [1] or drug delivery [2]. Chitin-based scaffolds of poriferan origin are able to absorb various liquids including water and blood [3,4].

In addition to chitin skeletons, sponges belonging to Verongiida order synthesize bioactive bromotyrosines and their derivatives known as multi-target marine drugs [5].

The aim of this study was to develop a novel method for the isolation of 3D chitin scaffolds simultaneously with bromotyrosine-containing extracts from Aplysina aerophopa, based on the use of ultrasonic treatment in methanol and sodium hydroxide solution.

Methanol dissolves organic compounds and can give high extraction yields. In addition, it is easy to evaporate.

This method is based on applying ultrasound during treatment in 200 ml of methanol with a 100 ml of 10% sodium hydroxide solution in 80°C. This approach takes about 3 hours and gives brominated chitin skeletons and extract containing bromotyrosines. The skeletons are then treated with water and acetic acid to dissolve inorganic salts. Finally, the skeletons are sonicated in 10% NaOH solution for approximately 4 hours in up to 55°C, resulting in white chitin scaffolds. This procedure does not deacetylate chitin to chitosan.

In contrast to known stepwise established extraction method proposed by Ehrlich [6], this ultrasonic approach is much less time-consuming.

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