XXIX Conference
Polish Chitin Society
Olsztyn
September, 18-20th, 2024

NEW ASPECTS ON CHEMISTRY AND APPLICATION OF CHITIN AND ITS DERIVATIVES

Łukasiewicz
Lodz
Institute of
Technology

UNIWERSYTET WARMIŃSKO-MAZURSKI W OLSZTYNIE

WYDZIAŁ GEOINŻYNIERII

XXIX Conference of Polish Chitin Society

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XXIXConference

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

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CONFERENCE SCHEDULE

September 18th 2024 – Wednesday

16^{00}-18^{00}	**Registration**
19^{00}-20^{00}	**Dinner**

September 19th 2024 – Thursday

9^{00}-9^{35}	**Opening ceremony** **Katarzyna Struszczyk-Świta, Ph.D., President of PTChit**
	Prof. Henryk Struszczyk prize - giving ceremony

9^{35}-10^{00}	**Plenary lecture**
	Samuel Hudson **A DISCUSSION OF THE SYNTHESIS AND CHARACTERIZATION OF CHITOSAN/PLASMID DNA NANOPARTICLES AND FUTURE CHALLENGES**

Session A		
Chairman		**Radosław Wach, Ph.D., D.Sc.**
A1	10^{00}-10^{25}	**Małgorzata M. Jaworska** **MODIFICATION OF CHITIN WITH IONIC LIQUIDS**
A2	10^{25}-10^{50}	**Anna Rył, Piotr Owczarz** **INFLUENCE OF INJECTION APPLICATION ON THE BULK AND LOCAL MECHANICAL PROPERTIES OF CHITOSAN HYDROGELS**
A3	10^{50}-11^{15}	**Krzysztof Nowacki, Maciej Galiński** **TANNIC ACID-CROSSLINKED CHITOSAN MEMBRANE AS A POLYMER MATRIX FOR THE HYDROGEL ELECTROLYTE: PRELIMINARY RESEARCH**
11^{15}-11^{35}		**Coffee/tea break**

Session B		
Chairman		**Anna Rył, Ph.D.**
B1	**11^{35}-12^{00}**	**Luminita Marin, Alexandru Anisiei, Daniela Ailincai, Sandu Cibotaru** **BIOABSORBABLE WOUND DRESSINGS BASED ON CHITOSAN NANOFIBERS: DESIGN, PHYSICO-CHEMICAL INVESTIGATION AND WOUND HEALING PERFORMANCE**
B2	**12^{00}-12^{25}**	**Magdalena Paczkowska-Walendowska, Judyta Cielecka-Piontek** **CHITOSAN-BASED MATERIALS FOR DENTAL APPLICATIONS**
B3	**12^{25}-12^{50}**	**Olga Marchut-Mikołajczyk, Piotr Drożdżyński, Katarzyna Struszczyk-Świta** **ENDOPHYTIC FUNGI – AN INTERESTING SOURCE OF CHITIN-MODIFYING ENZYMES**
12^{50}-13^{00}		**Magdalena Gierszewska** **PROGRESS ON CHEMISTRY AND APPLICATION OF CHITIN AND ITS DERIVATIVES"**
13^{00}-14^{00}		**Lunch**
14^{30}-19^{00}		**Visiting the monuments of Olsztyn with a guide**
20^{00}-24^{00}		**Gala dinner**

September 20th 2024 – Friday

9^{00}-10^{00}		**General Assemble of the Polish Chitin Society** *(only for PTChit members)*
Session C		
Chairman		**Katarzyna Małolepsza-Jarmołowska, Ph.D., D.Sc.**
C1	**10^{00}-10^{25}**	**Magdalena Gierszewska, Ewa Olewnik-Kruszkowska, Ewelina Jakubowska, Aleksandra Szydłowska-Czerniak** **CHITOSAN IN FOOD PACKAGING APPLICATIONS – PACKAGE FORMATION AND EXTENDING OF FOOD SHELF-LIFE**
C2	**10^{25}-10^{50}**	**Maria Stasińska-Jakubas, Katarzyna Rubinowska, Barbara Hawrylak-Nowak** **DIFFERENTIATED METABOLIC RESPONSE OF ST. JOHN'S WORT AND LEMON BALM TO THE FOLIAR APPLICATION OF CHITOSAN**

C3	**10^{50}-11^{15}**	**Klaudia Piekarska, Maria Wiśniewska-Wrona, Monika Owczarek, Monika Sikora, Dominik Borkowski, Piotr Drożdżyński, Katarzyna Struszczyk-Świta, Olga Machrut-Mikołajczyk, Grażyna Korbecka-Glinka** **ECOLOGICAL SOLUTIONS BASED ON CHITOSAN**
C4	**11^{15}-11^{40}**	**Małgorzata Gnus** **EXTERNAL MAGNETIC FIELD – EFFECT ON SEPARATION PROPERTIES OF CHITOSAN MEMBRANES CONTAINING MAGNETIC PARTICLES – COMPARISON OF ETHANOL DEHYDRATION PROCESS BY PERVAPORATION AND VAPOUR PERMEATION METHOD**
11^{40}-12^{00}		**Coffee/tea break**

Session P	**Poster session 12^{00}-12^{45}**
P1	**Urszula Filipkowska, Tomasz Jóźwiak, Malwina Nierobisz** **EFFICIENCY OF THE REACTIVE BLACK 5 DYE REMOVAL IN AN AIR-LIFT REACTOR AND IN A COLUMN REACTOR**
P2	**César I. Hernández Vázquez, Zbigniew Draczyński, Dominik Borkowski, Dorota Kaźmierczak** **ENHANCING CHITOSAN FIBERS: A DUAL APPROACH WITH TRIPOLYPHOSPHATE AND URSOLIC ACID**
P3	**Monika Owczarek, Maria Wiśniewska-Wrona, Wiesław Adamiec, Piotr Cichacz, Katarzyna Bartosik, Alicja Buczek** **PREPARATION IN FORM EMULSION BASED ON NATURAL POLYMERS WITH THE ADDITION OF ESSENTIAL OILS REPELLANT TO TICKS**
P4	**Jacek Nowaczyk, Aneta Jastrzębska** **EDIBLE MEAT PACKAGING BASED ON CHITOSAN**
P5	**Katarzyna Małolepsza-Jarmołowska, Hanna Bazan** **THE EFFECT CALCIUM ALGINATE ON THE NEUTRALIZING PROPERTIES OF CHITOSAN GELS PROTECTING THE ESOPHAGEAL MUCOSA**
P6	**Katarzyna Małolepsza-Jarmołowska, Hanna Bazan** **THE INFLUENCE OF HYDROPHILIZING SUBSTANCES ON THE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN GELS WITH A PROTECTIVE EFFECT ON THE ESOPHAGEAL MUCOSA**
P7	**Katarzyna Struszczyk-Świta** **MUCOR RACEMOSUS AND MUCOR HIEMALIS - SOURCES OF CHITINOLYTIC AND CHITOSANOLYTIC ENZYMES**
P8	**Renata Czechowska-Biskup, Piotr Ulański** **PHYSICOCHEMICAL PROPERTIES OF CHITOSAN WITH HIGH DEGREE OF DEACETYLATION FOR DIFFERENT SOLVENTS**

P9	**Piotr Drożdżyński, Katarzyna Struszczyk-Świta, Klaudia Piekarska, Olga Marchut-Mikołajczyk** **BIOPREPARATIONS BASED ON CHITOSAN AND BIOSURFACTANTS AS A PLANT GROWTH STIMULANT**
12^{45}-13^{00}	**Closing of the conference**
13^{00}-14^{00}	**Lunch**

Dawid Kasprzak[1,2]

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[2] *– Humboldt-Universität zu Berlin, Department of Chemistry, Brook-Taylor-Str. 2 12489 Berlin, Germany*

POWERING THE FUTURE: APPLICATION OF CHITIN-BASED MATERIALS FOR ENERGY STORAGE DEVICES

The increasing demand for energy-efficient solutions is evident across both industry and daily life. However, to align energy delivery systems with global ecological trends, it is crucial to reduce fossil fuel consumption and minimize environmental pollution. In response, the focus has shifted toward the development of sustainable and eco-friendly biopolymer-based energy storage devices, which hold the potential to revolutionize the way we store and deliver energy [1].

In this context, the presenter is excited to share his recent scientific breakthroughs in the use of chitin — a naturally abundant and biodegradable biopolymer — as a key material for developing innovative biopolymer-based components specifically tailored for integration into electrochemical power sources. His research primarily explores its application in high-performance and wearable supercapacitors and Zn-ion systems [2-6], which represent promising alternatives to conventional energy storage devices. By leveraging the unique properties of chitin, such as its mechanical strength, surface chemistry, and environmental sustainability, it is aimed to contribute to the creation of more efficient, durable, and eco-conscious energy storage solutions.

Acknowledgements:

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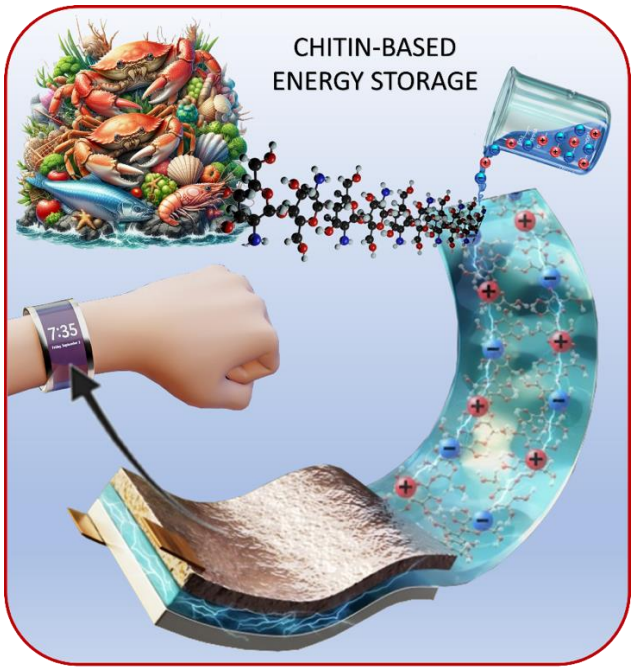

Figure 1. Illustration of the concept of chitin-based, sustainable and wearable energy storage devices.

OPENING LECTURE

Samuel Hudson
Chief Scientific Officer, Karamedica, Inc. Raleigh, NC USA

A DISCUSSION OF THE SYNTHESIS AND CHARACTERIZATION OF CHITOSAN/PLASMID DNA NANOPARTICLES AND FUTURE CHALLENGES

Chitosan readily forms nanoparticles which are being evaluated for a wide range of therapies, including gene delivery. The ionic formation of nanoparticles with plasmid DNA (for green fluorescent protein, GFP) and chitosan is discussed. The ability to synthesize chitosan-plasmid nanoparticles with controllable physical properties was assessed for chitosan and diethyl amino ethyl (DEAE) modified chitosan polymers. Controlling the diameter, surface chemistry and surface charge density are key to targeting the use of these chitosan based nanoparticles. Interestingly, chitosan and DEAE chitosan nanoparticles with similar physical properties were observed to have different size distributions, with chitosan particles having a much larger size distribution than DEAE chitosan nanoparticles.

Session A

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MODIFICATION OF CHITIN WITH IONIC LIQUIDS

Despite its unique properties (biodegradability, biocompatibility, and nontoxicity), chitin has limited application. The high crystallinity of chitin particles is the main reason against its application. Due to its high crystallinity, it is completely insoluble in most solvents; thus, heterogenic modifications of chitin can be performed mainly.

Ionic liquids (IL) are organic salts containing positively and negatively charged ions existing freely and stable in liquid form at temperatures below 100°C. The charge of the ion is mainly due to an imbalance of electrons (too many or too few) or due to dislocations of charge at the surface of an ion. Usually it is built with a large nitrogen-containing (eg. imidazolium) or phosphorus-containing cation (eg. phosphonium), while anion can be organic (eg. CH_3COO^-), or inorganic (eg. Cl^-, Br^-) and is much smaller. They have a low melting point, some of which are liquids at room temperature (so-called room temperature ionic liquids, RTILs). They are thermally stable and inflammable; the decomposition temperature is usually higher than 300°C. They are also reported as chemically stable and non-toxic.

It has been reported that some ILs are suitable solvents for chitin. The highest chitin solubility was reported from 3.5% w/w in [Emim][Cl] to 20% w/w in [Emim][OAc]. Such high solubility was useful for preparing chitin yards (by spinning or electrospinning), preparing scaffolds for stem cells, chitin composites with other biopolymers, or creating systems for water or CO_2 capture.

The aim of the work was to investigate the correlation between the structure of ionic liquid and the solubility of chitin and investigate changes in the structure of chitin after recovery from a solution in IL.

Several ionic liquids have been tested in our experiments. They can be divided into three groups:

- ionic liquids with imidazolium ring in a cation and with [Cl] anion: [Amim][Cl], [Emim][Cl], [Bmim][Cl], [Hmim][Cl], [Momim][Cl] and [Bdmim][Cl];
- ionic liquids with ethyl substituent in a cation ring and inorganic ([Cl], [Br], [I], [BFSI]) or organic ([Lact][OAc]) anion: [Emim][Cl], [Emim][Br], [Emim][I], [Emim][OAc], [Emim][Lact], [Epyr][I] and [EMS][BFSI].
- Ionic liquids with different rings in a cation ([MPpyrr], [MPpip], [DMPpz], [Emim]) and organic acid anion ([OAc], [Lact]) were tested.

Depending on which ionic liquid was used, different solubility, different modifications of the structure, or different particle sizes of chitin were observed.

We also proved that chitin can be entirely dissolved by ionic liquids containing organic acid anion and short (C1-C3) substituent in a cation ring. The highest concentration of chitin was observed in [Emim][OAc] (9%), but the solution was very viscous, which caused problems in handling.

The precipitation of ILs with water gave particles similar sizes and shapes when ionic liquids with inorganic anions were used. The larger particles and particles with the more expanded external surface area were obtained when ionic liquids with organic anion were used. That was probably due to the creation of new hydrogen bond networks between the chitin chains and the forming of large agglomerates.

No changes in the chemical structure of chitin were observed for the precipitated biopolymer, which suggested that no chemical bonds were created between chitin and the ionic liquid used.

The XRD analysis indicated that chitins after regeneration from ILs solution containing organic anion had smaller crystalline and crystallite size than untreated chitin used or chitin precipitated from the suspensions where ionic liquids with inorganic anion were used.

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INFLUENCE OF INJECTION APPLICATION ON THE BULK AND LOCAL MECHANICAL PROPERTIES OF CHITOSAN HYDROGELS

Thermosensitive chitosan scaffolds are a promising solution in the areas of tissue engineering and controlled drug delivery systems. This is due to their widely known biocompatible, biodegradable and non-toxic properties. Additionally, their ability to undergo a thermoinduced phase transition from a liquid sol form to a three-dimensional gel form allows for minimally invasive injection application, making this solution even more promising.

The assessment of the rheological properties of hydrogels for biomedical applications is extremely important because it allows the characterization of the mechanical properties of the biopolymer matrix both during compression (determination of Young's modulus value, texture analysis tests), tension and shear.

Due to the influence of injection application on the conditions of formation of the three-dimensional polymer network demonstrated by the authors [1], the aim of the work is to determine the influence of injection on the global and local mechanical properties of the obtained hydrogels, which has not been addressed in the literature so far.

Thermosensitive chitosan hydrogels were obtained according to the preparation proposed by Chenite et al. [2].

The bulk mechanical properties of the fabricated scaffolds were characterized based on mechanical spectra (frequency sweep tests) using an Anton Paar MCR 301 rotational rheometer equipped with a cone-plate measuring system. Additionally, texture profile analysis tests were conducted using the Brookfield CT3 testing machine.

In order to determine the local mechanical properties of biopolymer hydrogels, the dynamic light scattering (DLS) technique was used, which requires the suspension of inert, monodisperse polystyrene tracers of 500 nm in the tested system. Microrheological studies were performed using a Zeta Sizer Nano ZS90 analyzer according to the procedures proposed by Cai et al. [3]. Then, the raw data were analyzed using the Python programming language code developed by Cai et al. [3] to obtain the mechanical spectra.

In order to reproduce real injection conditions, injection needles in sizes 14G–25G were used, and a constant syringe plunger speed of 1 mm/s was ensured using a Harvard PHD2000 infusion pump.

Based on the analysis of the obtained mechanical spectra of chitosan hydrogels, an increase in the storage modulus G' value induced by the injection was observed. This increase also reflects a decrease in the average molecular weight of the polymer chain between neighboring crosslinks and a decrease in the network mesh size with a simultaneous increase in the crosslink density. The analysis of the texturometric test results allows for the formulation of a clear conclusion about the strong influence of the injection application on the hydrogel properties such as hardness, springiness, resilence, cohesion and adhesion, which is particularly important in the case of designing biomedical scaffolds because it determines the ability of cells to "stick" to the scaffold.

Based on the analysis of the microrheological tests carried out at a temperature of 37°C, it was found that the injection (capillary flow under high shear rates conditions) has a very strong influence on the determined values of the dynamic modulus G' on a microscopic scale. Regardless of the needle used to inject the polymer sol (before gelation), the dynamic modulus value is always lower than in the case of the control measurement (without gelation), which indicates a weakening of the structure of the prepared gel. At the same time, no correlation was observed between the needles used (shear rates during application) and the determined values of the storage modulus G'. This may be due to the non-ergodic state of the hydrogels.

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TANNIC ACID-CROSSLINKED CHITOSAN MEMBRANE AS A POLYMER MATRIX FOR THE HYDROGEL ELECTROLYTE: PRELIMINARY RESEARCH

As for today, the worldwide tendency to decrease greenhouse gas emissions and general air pollution forces gradual changes in our everyday lives, focusing mainly on replacing fossil fuels with alternative energy sources. Simultaneously, these environmentally friendly but weather-dependent sources of energy need efficient and reliable energy storage systems, mainly composed of electric double-layer capacitors (EDLCs) and batteries. Most of the traditional energy storage devices are filled with liquid electrolytes (mainly toxic organic salts), which generate leakage hazards and, therefore, cannot meet the newest strict environmental regulations. Thus, gel and hydrogel electrolytes are developed to overcome that issue and obtain high-safety electrochemical devices [1].

Hydrogel electrolytes are defined as polymer matrices with an aqueous electrolyte trapped inside the polymer chain interspaces. By using polysaccharides of natural origin (e.g., chitin, cellulose, chitosan) as a matrix, a promising alternative to conventional liquid electrolytes may be established [1,2].

Chitosan is widely used as a hydrogel material and can serve as a flexible and durable polymer matrix; however, this amino polysaccharide must be cross-linked to form dimensionally stable hydrogel with acceptable mechanical strength [2,3]. The covalent cross-linking of chitosan polymer matrices using an appropriate modifying agent (e.g., glutaraldehyde) or process conditions makes it possible to tailor the hydrogel properties. Nevertheless, that approach has drawbacks, such as reducing the polycationic character of chitosan by forming imine bonds. Therefore, a novel chitosan cross-linking system based on tannic acid, which may interact with chitosan polymeric chains by hydrogen bonds and thus preserve the unique composition of polysaccharide functional groups, was introduced [4,5].

Previously, chitosan-based hydrogel electrolytes were reported as components of EDLCs and exhibited good electrochemical properties, superior even to the devices with commercially available glass fiber separator [2,3]. Moreover, a significant influence of chitosan cross-linker type on the electrochemical performance of EDLC was also revealed [3]. Therefore, we hypothesize that tannic acid could be used as a replacement for toxic dialdehydes in the synthesis of chitosan-based hydrogel electrolytes. With this in mind, our research pursued aim to determine whether the fabricated chitosan-

based hydrogel electrolyte formed from tannic acid crosslinked chitosan matrix and reinforced with an aqueous solution of lithium sulfate has the potential to serve as a biodegradable and non-toxic-electrolyte for EDLC.

The prepared chitosan/tannic acid hydrogel electrolyte was used in EDLC cell to study its electrochemical properties. The electrochemical performance of the EDLC cell was determined by electrochemical impedance spectroscopy, cyclic voltammetry, and galvanostatic charging/discharging techniques. The results show that the EDLC cell with the investigated hydrogel electrolyte can be recognized as a fully functional and efficient device.

Acknowledgements:

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Session B

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BIOABSORBABLE WOUND DRESSINGS BASED ON CHITOSAN NANOFIBERS: DESIGN, PHYSICO-CHEMICAL INVESTIGATION AND WOUND HEALING PERFORMANCE

Background: Chitosan based nanofibers have emerged as promising biomaterials for tissue engineering, wound healing and hygiene related textiles, but their in vivo application is restricted by the lack of biodegradability of the synthetic polymers used as co-spinning agents. To overcome this issue, we designed and prepared fully biodegradable chitosan based nanofibers via electrospinning, when using poly(ethylene glycol) as sacrificial additive, as potential bioabsorbable wound dressings.
Methods: The composition and morphology of the fiber mats was confirmed by Fourier-transform infrared spectroscopy, proton nuclear magnetic resonance, thermogravimetric analysis, wide angle X-ray diffraction, polarized optical microscopy and scanning electron microscopy. In view of the targeted application, their properties, such as behaviour in moisture media (dynamic vapor sorption, swelling and enzymatic degradation tests), muco- and bio-adhesive character, mechanical properties and antimicrobial activity were investigated and the collected data were statistically analysed. The in vitro biocompatibility on normal human dermal fibroblasts was investigated in line with standards for biomedical devices and in vivo acute toxicity and biocompatibility was assessed by monitoring haematological, biochemical and immunological profile on Wistar rats. Wound closure and healing were studied on burn wound models in rats.
Results: The removal of the PEO co-spinning agent led to mesoporous chitosan nanofibers which favoured the improving of high swelling ability and fluid exchange, biodegradability, muco- and bio-adhesivity and mechanical properties. By modification with suitable bioactive agents, i.e. loading of antibiotics, surface modification by imination or using of quaternized chitosan in the electrospinning step, the fibers were endowed with strong antimicrobial activity against relevant pathogens, while preserving in vitro and in vivo biocompatibility. Moreover, their subcutaneous implantation in rats revealed in vivo biodegradation and lack of toxicity. As a proof of concept, the application

of the fiber mats in burn wound models in rats showed wound closure and active healing, with fully restoration of epithelia.

Conclusions: The use of poly(ethylene glycol) with double role, of co-spinning agent and sacrificial additive, is a straight pathway to the obtaining of chitosan based nanofibers, which design can be further elaborated towards bioabsorbable wound dressings which favours rapid wound closure and fully restoration of the skin tissue.

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CHITOSAN-BASED MATERIALS FOR DENTAL APPLICATIONS

Periodontal disease represents a significant global burden on oral health, with estimated prevalence ranging from 20% to 50% worldwide [1]. Recently, in the treatment of periodontitis, more and more attention is paid to the use of plant materials due to their multidirectional action profile. One such plant material is Scutellariae baicalensis radix (Baikal skullcap root) [2].

This research aims to prepare innovative pharmaceutical forms like powder systems, extrudates, mucoadhesive tablets as well as 3D-printed scaffolds as personalized dressing with proven anti-periodontal effect.

As a result of the application of the design of experiment (DoE) approach, it was possible to optimize the obtaining of an extract from S. baicalensis radix and prepare its systems with chitosan (70/80/90 DDA). The binary system containing lyophilized extract with chitosan 90:500 in weight ratio 2:1 was found to be most valuable with the appropriately controlled baicalin release, significant biological activity to inhibit the activity of the hyaluronidase enzyme, and appropriate mucoadhesive properties enabling prolonged residence time of the product at the application site.

Extrudates containing Scutellariae baicalensis radix extract with chitosan (CS 90/500 in weight ratio 2:1) show an interesting potential for improving the solubility of the poorly water-soluble active substance—baicalin. So, hot-melt extrusion is a good technique to improve the physicochemical properties of baicalin. Further, to obtain a suitable pharmaceutical form, the production process of mucoadhesive tablets containing extrudates was optimized. The prepared extrudates, differing in HPMC content, showed different tabletability, compressibility, and compactibility properties. As expected, the different content of the carrier influenced the release profile of baicalin from the tablets and the mucoadhesive properties. Higher HPMC content resulted in prolonged release of the substance, resulting from the diffusion of the substance through the polymer network. At the same time, the same carrier ensured that the tablets were kept in the affected area for a sufficiently long time. Importantly, the process did not reduce the biological, including microbiological, activity of the obtained extrudates. Considering the complex matrix, both the tabletability/compactibility properties of the blends and the degree of release of the active substance, as well as mucoadhesive properties that give functionality to the developed tablets, should be considered. The best tabletability properties, a valuable baicalin release

profile while maintaining sufficient mucoadhesive properties to condition the tablet's retention in the application site and the effectiveness of therapy, are provided by the formulation, which contains the extrudate with lyophilized extract-HPMC 50:50 w/w.

The obtained 3D dressings containing chitosan(HMW)/gelatin/extract systems meet the criteria for personalized treatment of periodontal diseases due to their functionality (proven anti-inflammatory effect) and form (preserving shape with long-term release of active compounds). The amorphous state of the 3D dressings containing chitosan/gelatin/extract systems was established by XRPD measurements, while the uniform distribution of hydrogel within the scaffold was proved by microscopic analysis. Furthermore, FTIR-ATR measurements defined interactions between the chitosan-based scaffold and the active compounds of the Baikal skullcap root extract. Their anti-inflammatory, microbiological, and wound healing activities, together with biocompatibility properties, ensure safety and biological effectiveness, supporting their potential in tissue engineering applications.

The proposed pharmaceutical forms based on chitosan constitute an innovative possibility of using plant materials, guaranteeing prolonged release of active substances and prolonged duration of active compounds remaining at the site of application, i.e. the diseased site, and constitute an alternative and significant improvement of treatment in relation to currently used gels and mouthwashes.

Acknowledgements:

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ENDOPHYTIC FUNGI – AN INTERESTING SOURCE OF CHITIN-MODIFYING ENZYMES

Endophytic fungi are a diverse group of microorganisms that inhabit the intercellular and intracellular regions of plant tissues, thereby having a beneficial impact on the host species. Due to their unique living conditions and ability to thrive in harsh environments such as high salinity and drought, along with their ability to utilize complex molecules as a carbon source, endophytic fungi can serve as a valuable source of enzymes with distinct features [1].

Chitin and chitosanolytic enzymes have unique properties that make them useful for making new chitosan oligosaccharides with a specific structure. These enzymes also help break down chitin waste, which is good for the environment because it lowers pollution. A better understanding of the structure and properties of chitin-modifying enzymes has led to an increased interest in their extraction from microbial sources and their use in the biotechnological production of these essential sugars [2].

Endophytic fungi's fascinating capacity to utilize processes that sequester or convert chitin present in the cell wall into its derivatives, allowing for suitable interactions with host plants, makes this taxonomic group a prospective source of chitin-modifying enzymes.

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Session C

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CHITOSAN IN FOOD PACKAGING APPLICATIONS – PACKAGE FORMATION AND EXTENDING OF FOOD SHELF-LIFE

According to the Mordor Intelligence™ Industry Reports, the size of the packaging market in 2024 is estimated at 1.14 trillion USD, and its value will increase by ca. 20% during the next 5 years. The primary role of food packaging is maintaining food safety and ensuring its quality during the supply chain. Chemical, biological, and physical protection against outside influences are the most important among the various roles food packaging plays. However, the package can also bring informative/marketing and traceability functions, reduce food damage and thus food waste, make food buying and processing more convenient, and finally inform consumers about the product's shelf life. The shelf-life of a food product is strongly affected by package design (e.g., the choice of packaging material) and package construction [1, 2].

The materials traditionally applied in food packaging include glass, metals (aluminum, foils and laminates, tinplate, and tin-free steel), paper and paperboards, and finally, plastics [1]. The two latter material types constitute ca. 70% of the total market. The contribution of the packaging sector to the annual global plastic waste generation last year was the highest among other industries, reaching ca. 142.6 mln tonnes, which constitutes ca. 45% of the total plastic waste production [3]. Thus, applying biodegradable substitutes, e.g., polysaccharides (gelatin, chitosan), can bring sustainability benefits and help reduce the packaging industry's environmental footprint.

There are several methods of chitosan-based package formation. Among mostly tested procedures, casting-solvent evaporation, direct coating (dip-coating, spread coating, spray coating), extrusion, electrospinning, and multilayered coating are mainly applied. Additionally, several strategies are proposed to improve the chosen properties of the chitosan package, i.e., its mechanical resistance and barrier properties, and to make the chitosan package active. The latest definition of active packaging stands that it "helps in maintaining or improving quality of processed and fresh foods, and ultimately enhances their shelf-life."

The research aimed to prepare a plasticized and modified chitosan package with various additives. The hypothesis was that the proposed modification would improve mechanical and antioxidative properties. The effect of developed chitosan materials on storing various food products (e.g., bread, strawberries, rapeseed oil) was examined. The conclusion regarding the influence of chitosan-based packages on extending shelf life was taken.

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DIFFERENTIATED METABOLIC RESPONSE OF ST. JOHN'S WORT AND LEMON BALM TO THE FOLIAR APPLICATION OF CHITOSAN

Chitosan is a biodegradable and biocompatible polysaccharide derived from the partial deacetylation of chitin. Its promising effects in optimizing plant production are closely linked to its ability to stimulate plant metabolic responses – this results in changes in the accumulation of specialized metabolites and can be used to modify plant phytochemistry in the desired direction [1].

The modulating effects of foliar applied chitosan lactate (ChL) on some aspects of primary and secondary metabolism in common and economically important medicinal plant species: St. John's wort (Hypericum perforatum L.) and lemon balm (Melissa officinalis L.) were compared [2, 3]. The experiments were carried out in a pot cultivation in a phytotron. The plants were treated with two foliar applications of an aqueous ChL solution (100 mg/L) at 10 mL/pot at three-day intervals. After 10 days from the first application of ChL, selected physiological and phytochemical parameters were analysed in the leaves of St. John's wort and lemon balm.

The concentration of total phenolic compounds in the leaves of ChL-treated plants varied depending on the species. The induction of accumulation of these compounds after ChL exposure in comparison to control plants was more efficient in the case of lemon balm (26% increase) than in St. John's wort (12% increase). The application of ChL also stimulated the biosynthesis of total soluble flavonols to a similar extent in both plant species, as the concentration increased by 50% and 35% in lemon balm and St. John's wort, respectively.

Due to the fact that the plant species compared belong to different taxonomic groups, phenolic metabolites dominated among the compounds analyzed in lemon balm, whereas mainly flavonoids were identified in St. John's wort. In the case of lemon balm, the accumulation of rosmarinic acid hexoside, rosmarinic acid and lithospermic acids was strongly enhanced by the ChL application. The levels of these phenolic acids increased even several fold compared to the control. The level of salvianolic acid was also stimulated by ChL treatment as its content was up to 3 times higher than the control. As a result of ChL application, the accumulation of caftaric acid and its hexoside also increased significantly – the concentrations of these metabolites were more than twice higher after ChL treatment than in the control plants [2].

The application of ChL also stimulated the biosynthesis of the flavonoids identified in St. John's wort, with a particularly positive effect on the contents of rutoside and quercetin – the levels of these flavonoids increased by 50% and 48%, respectively, compared to control plants. Simultaneously, the ChL treatment increased the hyperoside content in the St. John's wort leaves by approximately 29% above the control. However, although ChL foliar application generally increased the concentration of identified flavonoids, it did not induce the desired changes in the levels of naphthodianthrone or phloroglucinol metabolites [3].

No visual signs of toxicity of ChL were found, although there were some ChL-induced changes in parameters related to oxidative stress (increase in H_2O_2 and $O^{2\cdot-}$ accumulation and lipid peroxidation, modulation of the activity of enzymatic antioxidants) and photosynthesis (reduction in chlorophyll fluorescence indicators and photosynthetic pigment concentrations). These results suggest that foliar application of ChL can modulate the biosynthesis and accumulation of secondary metabolites in controlled pot cultivation of medicinal plants, thereby improving the quality of produced plant raw materials [2, 3].

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ECOLOGICAL SOLUTION BASED ON CHITOSAN

In the era of growing ecological awareness of society and concerns about the impact of chemical plant protection products on the environment and human health, modern plant protection products based on natural polymers are becoming increasingly popular, which is an alternative strategy with harmless substitutes for pesticides and a different mechanism of action [1]. Chitosan, as a well-known natural polymer, known for its bioactivity and biodegradability, has become a matrix for developing the discussed solutions [2]. Chitosan is also an elicitor, stimulates growth and supports the natural defense mechanisms of plants, indirectly affects the control of pests and plant diseases and has a positive effect on ecosystems. Preparations for supporting plant growth and protection have been additionally enriched with glycolipids from the endophytic strain of *Bacillus pumilus* 2A bacteria [3]. These products are biodegradable and do not leave toxic residues in soil, water or air. They can be used to protect ornamental plants, vegetables, fruits and other crops. The effectiveness and safety of chitosan-based plant protection products encouraged us to use an active layer on universal biodegradable mats for use as agrotextiles and seedling pots in agriculture, forestry and horticulture. The pots can be successfully used for hydroponic cultivation, they are biodegradable, support the growth of the root system and protect it from the negative effects of sudden changes in temperature, i.e. frosts, which can lead to thermal shock in the plant. Another ecological solution based on chitosan are repellents for ticks, mosquitoes, aphids and other insects. They are safe for humans and beneficial organisms in the ecosystem, while effectively deterring and protecting us from tick-borne diseases.

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EXTERNAL MAGNETIC FIELD – EFFECT ON SEPARATION PROPERTIES OF CHITOSAN MEMBRANES CONTAINING MAGNETIC PARTICLES – COMPARISON OF ETHANOL DEHYDRATION PROCESS BY PERVAPORATION AND VAPOUR PERMEATION METHOD

Particles with magnetic features provide good opportunities to develop materials with specialized properties. The magnetic field generated by such particles interacts with the surrounding matter, changing its properties. Modifying the structure of a composite material in the presence of an external magnetic field is a simple, non-invasive method of interfering with its structure and changing physicochemical and mechanical properties.

This work aims to determine how the content and distribution of ferrites in chitosan membranes influence their vapour transport properties. For this purpose, chitosan membranes containing different amounts of Fe_3O_4, $NiFe_2O_4$, $MnFe_2O_4$, and $MgFe_2O_4$ were examined. Magnetic particles were arranged in the membrane matrix by a perpendicular external magnetic field during the membrane preparation process. Transport properties of prepared membranes were tested in the ethanol dehydration process by vapour permeation method. The values were compared with results obtained earlier for the same membranes in the pervaporation process.

The results showed that the presence of ferrites in the polymer matrix affects membrane transport properties and depends on the filler content and their distribution. Moreover, the effectiveness of membranes is different in the pervaporation and vapour permeation process which is connected with the separation mechanism of used methods.

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POSTER SESSION

P1

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EFFICIENCY OF THE REACTIVE BLACK 5 DYE REMOVAL IN AN AIR-LIFT REACTOR AND IN A COLUMN REACTOR

The adsorption of the Reactive Black 5 dye from an aqueous solution in an air-lift reactor and in a column reactor was studied in two variants: using an unmodified chitosan sorbent and a chitosan sorbent modified with pentasodium tripolyphosphate. They allowed to determine the best conditions for the adsorption of coloured impurities from aqueous solutions. Under uniform flow conditions (50 cm^3/h) in the air-lift reactor and in the column reactor, the maximum adsorption capacities of the sorbents and the operating parameters of the tested reactors were determined. The RB5 adsorption process was the most efficient in the column reactor when using a sorbent in the form of non-crosslinked chitosan. The maximum adsorption capacity for the non-crosslinked chitosan sorbent was 941.6 mg/g in the air-lift reactor and 1249.0 mg/g in the column reactor. The maximum adsorption capacity for the chitosan sorbent cross-linked with pentasodium tripolyphosphate was 417.3 mg/g in the air-lift reactor and 382.1 mg/g in the column reactor.

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ENHANCING CHITOSAN FIBERS: A DUAL APPROACH WITH TRIPOLYPHOSPHATE AND URSOLIC ACID

Chitosan, a well-established biomaterial known for its biocompatibility, biodegradability, and bioactivity, has been the focus of extensive research in recent years. This study explores the enhancement of chitosan fibers'properties through wet impregnation with either ursolic acid (UA) or cross-linking with tripolyphosphate (TPP). In the first experiment, chitosan fibers were treated with UA, for varying immersion set points (1, 2, 4, 6, and 8 h). FTIR, SEM, and UV-Vis spectroscopy analyses demonstrated a chemical reaction between chitosan and UA, with stability reached after 2 h of immersion. Antibacterial testing revealed that chitosan fibers impregnated with UA exhibited significant antibacterial activity against Gram-positive bacteria, notably Staphylococcus aureus. The second experiment involved modifying chitosan fibers'surfaces with a 1% w/v TPP solution for the same periods of time (1, 2, 4, 6, and 8 h). Subsequently, the investigation involved FTIR, SEM, and dynamometry analyses, which revealed successful cross-linking between chitosan and TPP ions, resulting in improved tensile strength after 2 h of immersion. This dual-approach study highlights the potential of chitosan fibers for diverse applications, from wound-healing dressings to antibacterial materials against Gram-positive bacteria.

The results of the present study reveal the successful surface modification of 7% chitosan fibers through wet impregnation with ursolic acid and TPP, leading to distinct enhancements in antibacterial and mechanical properties, respectively. Ursolic acid modification primarily targets antibacterial enhancement, while TPP cross-linking focuses on mechanical improvements. The antibacterial properties of ursolic-acid-impregnated fibers demonstrate significant potential, particularly against Gram-positive strains such as Staphylococcus aureus.

The mechanical properties of the TPP cross-linked fibers were successfully enhanced, obtaining up to a 15% increase in tensile strength and up to a 9.75% increase in relative elongation. The potential application of these modified fibers

extends to wound dressings and scaffolds with improved antibacterial and mechanical characteristics. In future research endeavors, the exploration of elevated concentrations of ursolic acid represents a potential route for augmenting antibacterial efficacy, with a specific focus on enhancing resistance against Gram-negative bacterial strains. This aligns with the concept of optimizing the composition to achieve a broader antimicrobial effectiveness. Furthermore, a new approach entails examining the simultaneous improvement of both antibacterial and mechanical properties in a single sample, facilitating a comprehensive understanding of the dual-functional modifications.

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P3

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PREPARATION IN FORM EMULSION BASED ON NATURAL POLYMERS WITH THE ADDITION OF ESSENTIAL OILS REPELLANT TO TICKS

Ticks (mainly of the genus Ixodes) are one of the most dangerous arachnids that can threaten us from early spring to the end of autumn. A tick bite can be very dangerous, because it can transmit very dangerous microorganisms: the etiological factor of Lyme disease Lyme spirochetes (*Borrelia burgdorferi sensu stricto*, *B. garinii* and *B. afzelii*) and tick-borne encephalitis (TBE) by by the *Flavivirus* [1]. There are many preparations available on the market, for use directly on the skin in the form of sprays), spraying especially synthetic preparations, e.g. permethrin, cypermethrin or other pyrethroids (these are preparations harmful to human and animal health), as well as tablets and collars (for animals) [2]. Currently, there are no preparations available on the market based on natural polymers with the addition of effective repellants acting on ticks.

As part of the research work, active preparations (in liquid form) based on natural polymers (chitosan lactate) with the addition of selected compositions of essential oils acting repellently on ticks were developed. Solutions of natural polymers were used to develop preparations with a potential repellent effect. The oil phase consisted of essential oil compositions: tea tree oil, citronella, patchouli, clove and herbal aromas such as lavender, thyme, mint, oregano, lemon balm, rosemary, cinnamon, eucalyptus, geranium, lemongrass, marigold and others. The research methodology is based on emulsification methods, i.e. creating an oil-in-water emulsion. The emulsion preparations produced were evaluated in terms of physicochemical and rheological properties, biological properties and stability using the centrifuge and thermal.

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P4

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EDIBLE MEAT PACKAGING BASED ON CHITOSAN

The packaging sector has been looking for new solutions that align with the current trend toward biodegradable and renewable natural components. Active packaging and edible biopolymer packaging are two examples of the alternative solutions being considered in light of the drawbacks of biomaterial packaging. Designing edible food packaging provides one of the best possible solutions [1]. Extremely high standards are set for substances that can be safely eaten by people. In spite of first impressions, edible packaging for foods and medications is not a novel concept; in fact, it has been the subject of study for quite some time, with some solutions having made it to market [2]. There are a lot of materials that can't be utilized as the foundation for edible packaging due to legal limits and system regulations. In reality, these substances fall under a class of molecules that includes polysaccharides, proteins, lipids, and waxes.

In our work, we looked into the properties of edible packaging for poultry meat made of a particular emulsion formulation with an aqueous chitosan solution as a continuous phase.

Given its shown antibacterial activity and good gas barrier properties, chitosan has immense potential as a food packaging material [3]. Furthermore, it doesn't have any harmful effects on the body and breaks down naturally. Neat chitosan films are fragile and rigid, and they doesn't do a good job of preventing moisture from penetrating. In order to overcome this disadvantage we propose to include two phase system including hydrophilic chitosan matrix and hydrophobic oil inclusion. We found that adding a dispersed oil phase to chitosan-based films improved their mechanical properties. Furthermore, we assume that incorporating oil phase microdroplets into chitosan films not only will alter their mechanical properties but also will allow for the introduction of active compounds that are insoluble in water. This innovation could broaden the application of chitosan-based films as edible packaging and especially as an active packaging. In the given project we have investigated the storage and fundamental mechanical properties of films cast from solutions with different compositions.

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P5

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THE EFFECT CALCIUM ALGINATE ON THE NEUTRALIZING PROPERTIES OF CHITOSAN GELS PROTECTING THE ESOPHAGEAL MUCOSA

Research into methods of preventing damage to the esophageal mucosa due to reflux is still fully justified. The metods used so far do not bring fully satisfactory therapeutic effects. 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 [1-5].

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

The results of experimental work obtained in our own studies indicate the possibility of obtaining preparations that prevent damage to the esophageal mucosa resulting from gastroesophageal reflux. 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. Based on the obtained research results, the gels were enriched with the addition of calcium alginate. The introduction of this component allowed obtaining new beneficial properties of the tested gels. The most important aspect of this modification was the possibility of obtaining gels with a pH that allows neutralization of acid reflux. 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. Presented assumptions and investigations in vitro require verification in vivo, what is the aim of subsequent studies.

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THE INFLUENCE OF HYDROPHILIZING SUBSTANCES ON THE PHYSICOCHEMICAL PROPERTIES OF CHITOSAN GELS WITH A PROTECTIVE EFFECT ON THE ESOPHAGEAL MUCOSA

Agents that coat the esophageal mucosa and neutralize irritating contents and a used to relieve the effects of gastroesophageal reflux are washed out too quickly from the mucosal surface. Anatomical and physiological conditions make it difficult to effectively protect the mucosa from destruction by both acid and alkaline reflux [1-5].

The aim of the work was to investigate the influence of hydrophilizing substances on the adhesive properties of chitosan-containing gels.

During the experimental work, it was found possible to increase the adhesion of the tested gels due to the use of hydrophilizing substances in their composition. Increasing the adhesion of gels and, consequently, extending the time the preparation remains on the mucous membrane could significantly increase the effectiveness of the therapy. Prepared formulations were prepared with various rheological properties and adhesion work. An additional positive aspect obtained during the research is the proper pH of the preparations. On the basis of the tests, the dynamic viscosity and adhesion work 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. The pH range of the gels allows the selection of the optimal preparation. The research being conducted may help to accomplish the difficult task of long-term coverage of the esophageal mucosa and its effective protection against factors that irritate it and lead to chronic complications. Laboratory tests require clinical confirmation.

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MUCOR RACEMOSUS AND *MUCOR HIEMALIS* - SOURCES OF CHITINOLYTIC AND CHITOSANOLYTIC ENZYMES

Mucor is a fungal genus classified in the *Mucorales*, the most prominent order of zygospore-forming fungi. There is still growing interest for *Mucor* species due to some of their characteristic traits that have made them good models for genetic studies, but also for their importance as pathogens (including in humans), their contribution to fermented food production, cheese production and other biotechnological abilities [1]. *Mucor* species have important industrial applications due to their ability to produce a wide range of metabolites. They are a source of enzymes, including proteases, lipases, cellulases, chitinases and chitosanases, as well as lipids, pigments and biosurfactants [2].

Biotransformation of chitin to chitosan is carried out using chitin deacetylases (EC 3.5.1.41). Chitinases (EC 3.2.1.14) and chitosanases (EC 3.2.1.132) are also important enzymes involved in the hydrolysis of chitin and chitosan. The use of these enzymes is mainly related to the production of oligomers of the above-mentioned biopolymers (COS). This method is environmentally safe and allows obtaining products with a specific degree of polymerization (DP) and deacetylation (SD), which determine the biological properties of COS [3].

Previous studies conducted at the Institute of Molecular and Industrial Biotechnology of the Lodz University of Technology have shown that the *Mucor circinelloides* IBT-83 strain is a promising source of chitosan-hydrolyzing enzymes. Intracellular chitosanase isolated from this strain was partially purified and characterized. Moreover, it was proven that this strain produces intracellular chitin deacetylase [4-5]. The aim of this work was to characterize two other strains of the *Mucor* genus, i.e. *Mucor racemosus* and *Mucor hiemalis*, in terms of the production of chitin- and chitosanolytic enzymes. A method for partial purification of intracellular proteins from the above-mentioned strains was developed and their activity towards chitin and chitosan hydrolysis was confirmed.

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PHYSICOCHEMICAL PROPERTIES OF CHITOSAN WITH HIGH DEGREE OF DEACETYLATION FOR DIFFERENT SOLVENTS

Chitosan is a linear polysaccharide obtained by chemical deacetylation of chitin. It is bioactive and biodegradable, and thus it has a wide range of applications. Recently, there is a growing number of studies on the use of chitosan in drug delivery systems, in synthesis of gold nanoparticles stabilized by chitosan and in additions to the biodegradable packing. Physicochemical properties of chitosan significantly depend on an origin of raw material (krill, shrimp or fungi chitin) and the processing methods. Products of various origins are characterized by different proportions of chitin to chitosan units (fraction of chitosan units is called a degree of deacetylation (DA)), different distributions and values of average molecular weights as well as a solubility. It is obvious that these factors have influence on chitosan properties and its biological activity, and therefore there is a need for methods and procedures for precise, fast and convenient determination of these parameters.

Typical solvents used in determination of average molecular weight of chitosan (viscometric and by light scattering) or for studying its physicochemical properties in solutions consist of three components: water, acid (to protonate the amino groups and dissolve chitosan) and salt (to screen the electrostatic forces between the cationic groups and thus provide the desired coiled conformation of macromolecules) (1,2). In this work we chose a few popular solvents and our proposed solvent for chitosan and we compare physicochemical behaviours of chitosan in these solvents.

For chitosans of DA ca. 85%, in the chosen range of acid concentrations (0.1 – 0.3 M HCl), values of viscosity and macromolecular size (Rg) are very similar to those obtained in systems of the same ionic strength, consisting of stoichiometric amount of acid and added salt (HCl + NaCl). Experimental values of Rg/Rh parameter (where Rh denotes the hydrodynamic radius) also confirm coiled conformation of chitosan in the tested solvents. The Mark-Houwink parameters

have been determined for chitosan in our proposed solvent, i.e. aqueous solutions of hydrochloric acid.

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BIOPREPARATIONS BASED ON CHITOSAN AND BIOSURFACTANTS AS A PLANT GROWTH STIMULANT

In recent years, there has been a growing interest in the agriculture sector about the application of organic fertilizers. A suitable selection of biopolymers can be employed for the production of ecological plant protection products. The antibacterial properties of chitosan make it viable for agricultural applications [1]. Nevertheless, this biopolymer can also function as a transporter for other phytochemically active substances, such as biosurfactants. The use of chitosan as a carrier for other compounds enables the production of preparations with diverse characteristics, facilitating the reduction of chemically manufactured plant protection products and the incorporation of supplementary features into soil, such as promoting plant growth [2].

The objective of this study was to provide a contemporary human and environmentally sustainable product for optimal plant development and preservation. The findings of our study shown that the combination of chitosan and biosurfactants stimulated the development of cress, both in its root and stem, which is employed as an indicator plant. A fourfold increase in root growth and an eightfold increase in stem growth were seen in comparison to the control sample, which consisted of water-saturated soil.

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