BUTYRIC-ACETIC CHITIN COPOLYESTER (BAC) – THE JOURNEY FROM CHITIN TO DRESSING MATERIAL ENHANCING WOUND HEALING

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ABSTRACT
The paper presents a method of chitin chemical modification leading to the butyric-acetic chitin copolyester (BAC) with properties that predispose it to applications as a wound-healing dressing. The paper shows the possibility of using chitins of various origins for the production of ester derivatives. The possibility of using chitin obtained from honeybees, krill and crab has been shown. The BAC was obtained by esterification of chitin with a mixture of butyric and acetic anhydrides. A method for producing fibers and flat porous materials for medical applications from this polymer is presented. The results of biological tests of the produced BAC structures are also shown.

Key Words: chitin, butyric-acetic chitin copolyester, biodegradation, wound healing

1. INTRODUCTION

Since the end of the eighties of the last century, the Department of Materials Science, Commodity Science and Textile Metrology of the Lodz University of Technology is implementing a research program whose main goal is to develop a methodology for the production of materials for medical applications based on chitin. Chitin is the second in terms of occurrence in nature biopolymer - polycarbohydrate, produced mainly by crustaceans, insects, arachnids. Chitin also occurs in the cell walls of fungi, seaweed and bacteria. In all living organisms it is combined in complexes with proteins and other polymers produced by these organisms. It is estimated that about 10 Gigaton of chitin is produced every year on the globe. Produced by living organisms, chitin has unique biological properties, from ancient times it is known as a material, spontaneously accelerating the healing of numerous wounds, and has been used in the form of powdered shellfish shells for their treatment.

Chitin is a high molecular weight linear polymer composed of N-acetylglucosamine matrices, specifically from 2- (acetylamino) -2-deoxy-D-glucose units linked by β-glycoside bonds at the 1,4 position. In the classic two-step process of obtaining chitin from crustaceans, the first stage is the removal of mineral salts by treatment with hydrochloric acid most often at a concentration of 0.2 to 2 mol/dm^3 in the temperature range from 0 to 100 °C. The second stage is the treatment with aqueous alkali solutions, mostly NaOH at a concentration of 1 mol/dm^3, aimed at removing the protein substances present in the shells. In the scientific literature one can find reports on the next stage of chitin purification, removal of pigments related to chitin (melanin). Methods of removing pigments with ethanol or ether [1], solutions of sodium hypochlorite [2,3], anhydrous acetone [4,5], chloroform [6], hydrogen peroxide [7,8] or also ethyl acetate [17] are described. However, there are opinions that organic solvents are not as effective in removing pigments as sodium hypochlorite or hydrogen peroxide [3,10].

The aim of this study was to show the potential and opportunities in the production of chitin dressing materials to accelerate the wounds healing.

2. CHITIN FORM HONEY BEE

Chitins from various sources were used in our studies. One of them were insects, bodies of naturally dead honey bees. The chitin isolation and purification processes developed by our team may provide the basis for using this source to obtain chitin on an industrial scale.

Chitin from honeybee bodies used in the work was obtained according to the developed, published [11,12] multi-stage chemical treatment process of the raw material supplied by beekeepers. The developed conditions for conducting each of these stages have a significant impact on the amount and physicochemical properties of the finally obtained chitin.

Based on the conducted research, the typical process of obtaining chitin from this source can be divided into the following stages:

a) Removal of waxes by Soxhlet extraction using ethanol. The use of ethanol in the extraction of wax substances covering the body of bees, allows their use for other purposes.
b) Removal of mineral salts using 10 times excess of 1 mol/ dm^3 HCl acid at 25 °C. By using a mechanical mixing of the resulting suspension over a period of 2 hours, a product containing no calcium and no magnesium salts was obtained.
c) Removal of protein substances using a 15-fold excess of NaOH solution at a concentration of 1 mol/dm^3 at 80 °C. After 40 hours of treatment, the product was a chitin complex with melanin with a dark brown color. It is characterized by a high content of the soluble part in a typical chitin solvent (DMAc/5% LiCl) while maintaining sufficiently high viscosity values of the chitin solution.
d) Removal of melanin was carried out by means of a redox reaction. As the optimal conditions for this process, the use of dilute aqueous solutions of manganese (VII) potassium (KMnO4) at a concentration of 0.1% used in a 10-fold excess for 60 min at 25 °C was determined. After oxidation of melanin, a reducing treatment using 1% oxalic acid was used.

Chitin obtained according to the above procedure is characterized by high molar mass values. Viscosimetric average molar mass Mv determined for obtained chitin was 430 kg/mol.

3. BAC SYNTHESIS WITH ACIDIC KATALYSTS

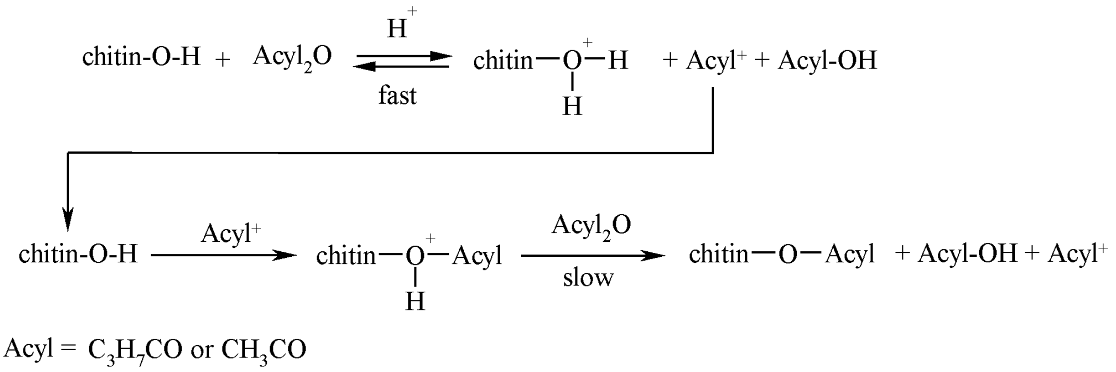
The developed conditions for the synthesis of copolyesters of chitin containing simultaneously in the macromolecule next to the side groups of butyric acid residues also acetic acid residue. This construction allows to obtain a differentiation of the size of side groups present on the chitin sugar chain.

This differentiation allows to reduce the distance between macromolecules, which in turn increases the number of intermolecular hydrogen bonds contributing to the increase of the mechanical strength of the obtained forms. A synthesis was carried out using two different methods:

- The first using perchloric acid as a catalyst (conducting synthesis in heterogeneous conditions).
- The second using methane sulfonic acid as a catalyst and solvent (conducting the synthesis in homogeneous conditions).

The basis for the selection of the synthesis butyrıc-acetıc chıtın copolyester were results confirming the required, predetermined chemical compositions of the esterification products with the assumed molar masses, presented by the value of intrinsic viscosity. The next parameter describing the synthesis was process efficiency [13].

Synthesis of butyrıc-acetıc chıtın copolyesters can be presented according to the following scheme (scheme 1)



Scheme 1. Synthesis of butyrıc-acetıc chıtın copolyesters [14]

Table 1. Physicochemical properties of butyrıc-acetıc chıtın copolyester containing 90% butyric acid residues and 10% acetic acid residues

Catalyst	The degree of acetylation	Contribution of butyric groups	Contribution of acetic groups	Part of the soluble fraction in ethanol [%]	Intrinsic Viscosity [dL/g]
$HClO_4$ CAS 7601-90-3	2.05	1.81	0.24	81	2.0
CH_4O_3S CAS 75-75-2	2.00	1.80	0.2	95	1.83

The reaction time - 4 hours, temperature 20 °C, the proportion of acid residue groups was determined by ^{1}H NMR spectra.

Based on an example of a copolyester containing 90% of butyric acid residues and 10% of acetic acid residues obtained both by perchloric acid catalyzed (heterogeneous conditions) or methane sulfonic acid (under homogeneous conditions), the proposed reaction allows obtaining a product with the required chemical composition and required molar mass. The results of research conducted on various chitin sources indicated that regardless of the source of chitin, the esterification products have an analogous chemical structure, which allows the use of chitin from any source, provided that the appropriate physicochemical parameters are met.

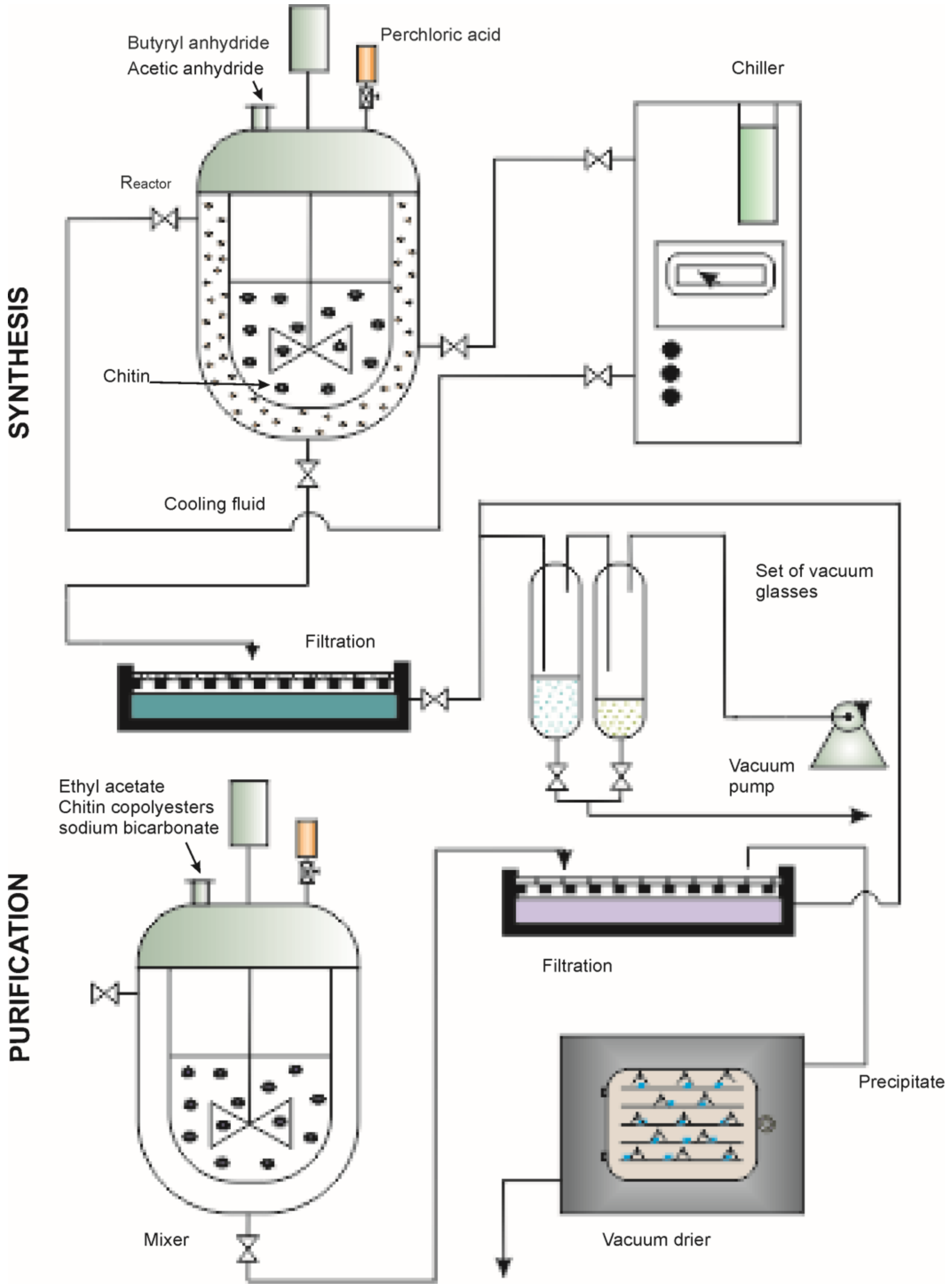
4. UPSCALING THE SYNTESIS

Conducted studies on the synthesis of butyrıc-acetıc chıtın copolyester, containing various proportions of butyric acid and acetic acid residues, met with the interest of the industrial partner. The Tricomed SA Company, Poland together with the team of the Lodz University of Technology has made an attempt to scale up to industrial conditions, the synthesis of a butyrıc-acetıc chitin copolyester containing besides butyric groups also acetic groups [14].

Transferring the scale of synthesis from the laboratory scale to the industrial scale does not only involve a simple increase in the amount of reagents used and the use of larger reactors.

It carries a great risk, such as the problem of transporting anhydrides, introducing a catalyst, maintaining the reaction temperature regime. Therefore, an attempt was made to transfer the scale to a partner with an established position on the market and having previous experience in adapting the synthesis from the laboratory scale to the industrial scale.

As a result of cooperation with the company Tricomed SA, the goal of transferring the synthesis from the laboratory scale to the industrial scale has been achieved. As a result of the research on esterification products, the following process diagram was determined.



Scheme 2. An industrial path of synthesis of butyric-acetic chitin copolyester [14]

The carried out comparative tests of butyric-acetic chitin *co*-polyesters with a molar ratio of 9 : 1 butyric acid residues to acetic acid residues (BAC 90/10) synthesis at laboratory and industrial scales showed that, after dealing with a significant number of problems of technical nature, it was possible to obtain in industrial conditions the polymer of exactly the same physicochemical parameters.

5. POROUS WOUND CHEALING MATERIALS

The butyric-acetic chitin co-polyester with a molar ratio of 9: 1 butyric acid residues to acetic acid residues (BAC 90/10) was used for the process of forming flat porous dressings. The method of washing out the blowing agent in the form of salt crystals was adopted for the manufacture of dressings. For the production of dressings with high porosity, salts of organic and inorganic acids insoluble in ethanol, which was a copolyester solvent, were used. The selection criterion was the high solubility of the salt in water as well as the size of the crystals. For forming flat porous films, salts whose lateral dimension did not exceed 0.4 mm were used [15,16].

Table 3 shows the list of salts used and average pores as well as total porosity of formed wound healing materials.

Table 3. The results of middle-sized pores and total porosity for manufactured membrane with the use of various inorganic salts.

Porophor agents	Organic solvent	Average pores diameter	Total porosity
		μm	%
Potassium carbonate	ethanol	270	96-98
Potassium bicarbonate	ethanol	260	96-98
Potassium bisulfate	ethanol	300	95-98
Potassium nitrite	ethanol	270	96-98
Ammonium carbonate	ethanol	250	96-98
Ammonium bicarbonate	ethanol	280	97-99
Di-ammonium hydrogen citrate	ethanol/chloroform	320	96-99
Di-ammonium oxalate 1-hydrate	ethanol/chloroform	340	95-98
Sodium carbonate 10-hydrate	ethanol/chloroform	250	96-98
Sodium carbonate	ethanol	280	96-98
Sodium bicarbonate	ethanol	250	96-99
Sodium chloride	ethanol	290	95-98

The mechanical tests showed that for all manufactured high porous films the value of tensile strength for samples about dimensions 2 cm wide and 0.11 thick was not more than 5cN.

Figure 1. Manufactured porous material from acetyl-butyryl chitin co-polyester (90/10) BAC and NaCl as the porophor agent in the membrane form [15]

Dressing material presented in figure1 was manufactured according to the salt-leaching method with the use of acetyl-butyryl chitin co-polyester (90/10) with the viscous volume 1.4 dl/g, sodium chloride with molecule diameter 0.16-0.40 nm and micro-silver particle.

Salt-leaching method for forming high porous dressings from as a example the acetyl-butyryl chitin co-polyester (90/10) leads to obtain the porous materials which pores shape is modelled on used salt crystals. Obtained materials have confirmed safety for using as a materials supporting wound healing process [16].

6. SUMMARY

The use of chitin for the synthesis of a new ester derivative of butyrıc-acetıc chıtın copolyester with a predetermined proportion of acidic residues makes it possible to use this material as a new material for biological applications. The conducted research has shown that there is the possibility of using chitin from sources such as insects, crustaceans as an excellent substrate for the synthesis of ester-derived chitin, which is soluble in typical solvents. The indicated synthesis methods have the ability to transfer them to the industrial scale. The produced porous materials are an excellent material for dressings that support the wound healing process without the need to replace them during the process of quenching. Dressings made from these derivatives have resorption properties by enzymes present in wound exudate.

7. REFERENCES

1. Hackman R.H. “Studies on chitin. I. Enzymatic degradation of chitin and chitin esters” *Australian Journal of Biological Sciences*,1954, **7**, 168
2. Blumberg R., Southall C.L., Van Rensburg N.J. and Volckman O.B. “South African fish products. XXXII-The rock lobster: a study of chitin production from processing wastes” *Journal of the Science of Food and Agriculture*, 1951, **2**, 571
3. No H.K., Meyers S.P. and Lee K.S. “Isolation and characterization of chitin from crawfish shell waste” *Journal of Agricultural and Food Chemistry*, 1989, **37**, 575
4. Kamasastri P.V. and Prabhu P.V. “Preparation of chitin and glucosamine from prawn shell waste” *Journal of Scientific and Industrial Research*, 1961, **20D**, 466
5. Mima S., Miya M., Iwamoto R. and Yoshikawa S. “Highly deacetylated chitosan and its properties” *Journal of Applied Polymer Science*, 1983, **28**, 1909,
6. Anderson C.G., de Pablo N.and Romo C.R. “Antarctic krill (Euphausia superba) as a source of chitin and chitosan” R.A.A. Muzzarelli, E.R. Pariser, Editors , Pr*oceeding of the first international conference on chitin/chitosan*, MIT Sea Grant Program, Cambridge ,1978, 54
7. Brine C.J. and Austin P.R. “Chitin variability with species and method of preparation” *Comparative Biochemistry and Physiology*, 1981, **69B**, 283
8. Moorjani M.N., Achutha V. and Khasim D.I. “Parameters affecting the viscosity of chitosan from prawn waste” *Journal of Food Science and Technology*, 1975, **12**, 187
9. Brzeski M.M. “Concept of chitin/chitosan isolation from Antarctic krill (Euphausia superba) shells on a technical scale” S. Hirano, S. Tokura, Editors, *Proceedings of the second international conference on chitin and chitosan, The Japan Society of Chitin and Chitosan*, Sapporo, Japan, 1982, 15
10. H.K. No and M.Y. Lee, “Isolation of chitin from crab shell waste” *Journal of the Korean Society of Food and Nutrition*, 1995, **24,** 105,
11. Draczynski, Z. “Honeybee corpses as an available source of chitin” *Journal of Applied Polymer Science*, 2008 **109** (3) , 1974
12. Nemstew S.V., Zueva O. Yu., Kihsmatullin M.R., Albulov A.I., Varlamov V.P. “Isolation of chitin and chitosan from honeybees” *Applied Biochemistry and Microbiology*, 2004, **40**, No.1, 39
13. Draczynski, Z. “Synthesis and solubility properties of chitin acetate/butyrate copolymers” *Journal of Applied Polymer Science*, 2011, **122**, 175
14. Draczynski, Z, Bogun, M, Sujka, W, Kolesinska, B. “An industrial-scale synthesis of biodegradable soluble in organic solvents butyric–acetic chitin copolyesters” *Adv Polym Technol*. 2018; 37: 3210–3221
15. Draczynski, Z.; Kolesinska, B.; Latanska, I.; Sujka, W. Preparation Method of Porous Dressing Materials Based on Butyric-Acetic Chitin Co-Polyesters. *Materials* 2018, 11, 2359
16. Sujka, W.; Draczynski, Z.; Kolesinska, B.; Latanska, I.; Jastrzebski, Z.; Rybak, Z.; Zywicka, B. Influence of Porous Dressings Based on Butyric-Acetic Chitin Co-Polymer on Biological Processes In Vitro and In Vivo. *Materials* **2019**, *12*, 970