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Synthesis and physicochemical characterization of chitin dihexanoate — A new biocompatible chitin derivative — In comparison to chitin dibutyrate

Karolina Skołucka-Szary [a,*], Aleksandra Ramięga [a], Wanda Piaskowska [a], Bartosz Janicki [b], Magdalena Grala [c], Piotr Rieske [a], Zbigniew Bartczak [c], Sylwester Piaskowski [a]

[a] Department of Research and Development, Celther Poland Sp. z o.o. ul. Ostrzykowizna 14A, 05-170 Zakroczym, Poland
[b] Silesian University of Technology, Faculty of Chemistry, Department of Physical Chemistry and Technology of Polymers, ul. M. Strzody 9, 44-100 Gliwice, Poland
[c] Centre of Molecular and Macromolecular Studies, Polish Academy of Sciences, Sienkiewicza 112, 90-363 Lodz, Poland

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ABSTRACT

Chitin dihexanoate (DHCH) is the novel biocompatible and technologically friendly highly substituted chitin diester. Here we described optimization of DHCH and chitin dibutyrate (dibutyryl chitin, DBC) synthesis conditions (temperature and reaction time) to obtain desired polymers with high reaction yield, high substitution degree (close to 2) and appropriately high molecular weights. A two-step procedure, employing acidic anhydrides (hexanoic or butyric anhydride) as the acylation agent and methanesulfonic acid both as the catalyst and the reaction medium, was applied. Chemical structures of DBC and DHCH were confirmed by NMR (^{1}H and ^{13}C) and IR investigations. Mechanical properties, thermogravimetric analysis, differential scanning calorimetry and biocompatibility (Neutral red uptake assay, Skin Sensitization and Irritation Tests) were assessed. Both polymers proved highly biocompatible (non-cytotoxic in vitro, non-irritating and non-allergic to skin) and soluble in several organic solvents (dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, acetone, ethanol and others). It is worth emphasizing that DHCH and DBC can be easily processed by solvent casting method and the salt-leaching method, what gives the opportunity to prepare highly porous structures, which can be further successfully applied as the material for wound dressings and scaffolds for tissue engineering.

1. Introduction

Chitin, poly(β-(1 → 4)-N-acetyl-D-glucosamine) is second only to cellulose as the most abundant natural polymer in the world. It is structurally similar to cellulose, from which it differs by having the acetamide groups ($-NHCOCH_3$) at C-2 position of N-acetylglucosamine units. It has been estimated that 10^{10}–10^{12} tons of chitin is biosynthesized each year [1]. Chitin is the principal structural polysaccharide of arthropods (mainly crustaceans [2], insects [3] and arachnids [4]), the mollusk shells [2] and the cell walls of fungi [5,6]. The recent scientific reports indicate that chitin can be also found in marine and freshwater sponges [7,8], corals [9] and in cell walls of diatoms [10]. Due to its physical and chemical properties chitin and its derivatives are being used in a vast array of different products and applications ranging from cosmetics and pharmaceutics to extreme biomimetics [11,12], food, agriculture, textiles and wastewater treatment [13–15]. Owing to advantageous biological properties such as biocompatibility, biodegradability, hemostatic activity and the ability to improve the wound healing process are well known [16–18], chitin can be considered as an excellent material for medical and pharmaceutical application [19], especially for wound dressing materials, artificial skin substitutes [20,21] and in tissue engineering field [22]. Chitin molecules demonstrate the tendency of the inter- and intra-molecular association (by means of inter- and intra-chain hydrogen bonds) with the formation of highly oriented supramolecular structure leading to its limited solubility in common organic solvents [23–27]. α-chitin is soluble only in several solvents such as aqueous thiourea, alkaline aqueous urea, dimethyloformamide/LiCl system, dimethylacetamide/LiCl system, ionic liquids [28–30], some fluorinated solvents such as hexafluoroacetone, hexafluoro-2-propanol, hexafluoroisopropyl alcohol [31,32], methanesulfonic acid [33–35], trichloroacetic acid, dichloroacetic acid systems [32] and in few other comparably toxic and corrosive solvents. In contrast to α-chitin, β-chitin is characterized by a higher affinity to water and organic solvents (soluble in formic acid) due to its weak intermolecular hydrogen bonding [24]. Consequently, because of its poor solubility, chitin has received little industrial attention. The presence of free hydroxyl groups in the chitin structure opens a possibility of its chemical modification that can lead

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* Corresponding author at: Celther Polska Sp. z o.o., Ostrzykowizna 14A, 05-170 Zakroczym, Poland.
E-mail address: karolina.skolucka@celther.com (K. Skołucka-Szary).

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to the development on new chitin derivatives with potentially distinctive biological properties. It was reported that the substitution of the hydroxyl groups in the chitin chain with ester groups may weaken the intermolecular hydrogen bonding and result in its better solubility in common organic solvents [36–41].

O-acylation with fatty acids is an effective method of improving the solubility of chitin for subsequent application. The esterification process requiring the use of suitable catalyst such as methanesulfonic acid or perchloric acid yields formylated [36], propionylated [36], butyrylated [36,38,41], valerylated [39] chitins and mixed esters (e.g. chitin co-(acetate/propionate), chitin co-(acetate/butyrate)) [23,40]. Alternative procedures include the synthesis of mixed/single chitin esters with fatty acids in trifluoroacetic anhydride (chitin co-(acetate/butyrate), chitin co-(acetate/hexanoate), chitin co-(acetate/octanoate), chitin co-(acetate/palmitate) and single chitin esters such as chitin acetate, butyrate, hexanoate and octanoate) [37] or esterification with butyric acid in the presence of trifluoroacetic anhydride/phosphoric mediated system (chitin dibutyrate) [38]. Depending on the degree of substitution, the O-acyl chain length and the content of acetate group in chitin copolymers, the resulting chitin esters, diesters and mixed esters can either be soluble or soluble to some extent in organic solvents, what makes them easily processable by simple techniques like solvent casting [39, 40], leaching [39] or wet/dry spinning [42,43].

The aim of the hereby presented work was to develop an effective method of synthesis of chitin dihexanoate – a new biocompatible and technologically attractive (easily soluble in organic solvents) chitin diester – and compare its physicochemical and basic biological properties with chitin dibutyrate, the well-known chitin derivative. Due to the fact that the chitin O-acylation system consisting of hexanoic acid and trifluoroacetic anhydride allows obtaining a chitin derivative only with low substitution degree [37], the proposed method of chitin dihexanoate synthesis is based on a method of synthesis of dibutyryl chitin known from the state of art, employing the methanesulfonic acid as the catalyst and the reaction medium [36]. This work is the continuation and complement to chitin diesters investigations, which were presented in our previous study [39].

2. Experimental

Research methodology (methods presented below) used for chitin dihexanoate and chitin dibutyrate characterization is based on the methodology described in the previous paper [39].

2.1. Synthesis of chitin dihexanoate (DHCH) and chitin dibutyrate (dibutyryl chitin, DBC)

Hexanoic anhydride (97%, Sigma Aldrich), butyric anhydride (98%, Sigma Aldrich), methanesulfonic acid (99%, ACROS Organics), acetone (pure, Avantor Performance Materials Poland S.A.), dimethylacetamide (DMAc, pure, Avantor Performance Materials Poland S.A.), LiCl (anhydrous, pure, Avantor Performance Materials Poland S.A.), $NaHCO_3$ (pure, Avantor Performance Materials Poland S.A.), hydrochloric acid (35–38%, pure, Avantor Performance Materials Poland S.A.) were used without further purification. Krill chitin α-type (France Chitine, Orange, France, degree of acylation of 0.97 calculated based on IR analysis, intrinsic viscosity value of 14.16 dl/g determined in DMAC/5% LiCl at 25 °C and viscosity average molar mass of 291,744 Da) was purified from residual calcium carbonate and other mineral residues. The purification step comprised of treating chitin with 2 M HCl for 2 h, followed by washing with distilled water to pH 7, filtering and eventually drying in a desiccator.

Chitin dihexanoate (DHCH) and chitin dibutyrate (DBC) were both synthesized by means of the two-step method, employing methanesulfonic acid as both the catalyst and the reaction medium. In first step of the synthesis, the reaction mixture was prepared. Aliquots of 15.3 ml and 9.6 ml of methanesulfonic acid were added dropwise to hexanoic anhydride (22.2 ml) and butanoic anhydride (16.2 ml) kept in a cooling bath at the temperature ranging from −5 to 5 °C while stirring, respectively. In both cases, the chitin acylation step and raw product purification step were the same. Small portions (3 g) of purified chitin were added step by step to freshly prepared acylation mixture kept at the temperature around 0 °C and continuously stirred for about 15 min using anchor-shaped stirrer equipped with flow baffles. Each synthesis was carried out under conditions (temperature, time) provided in Table 1. Next, reaction mixtures from all runs were neutralized using aqueous solution of 4% $NaHCO_3$ with simultaneous homogenization with an anchor-shaped stirrer equipped with flow baffles (fragmentation of reaction mixture). The obtained raw products were washed with water to pH 7, filtered and dried. The raw products were dissolved in acetone, filtrated to separate the unreacted chitin, precipitated with distilled water, and dried. Final products were dried in vacuum.

Characteristic parameters including yield of the reaction, intrinsic viscosity, average molecular weight and molecular weight distribution of obtained products were determined and measured in the function of reaction conditions. Each synthesis was repeated 5 times.

2.2. Characteristics of samples of chitin and chitin diesters

Intrinsic viscosity: The intrinsic viscosity [η] of chitin was determined in DMAC/5%LiCl system at 25 °C using the Ubbelohde viscometer. Chitin viscosity average molar mass M_v was calculated using the Mark–Houwink equation [30] $[\eta] = 0.0024 \times M_w^{0.69}$. The chitin acetylation degree was determined employing the method described in the literature [44,45] based on the analysis of infrared spectrum according to equation $DA\ (\%) = (A_{1655}/A_{3450}) \times 115$, where A_{1655} and A_{3450} stand for the absorbance of amide I peak (analytical band, 1655–1650 cm^{-1}) and the absorbance of hydroxyl peak (reference band, at 3450 cm^{-1}), respectively.

The coefficients of intrinsic viscosity [η] of obtained samples of chitin diesters were determined in acetone solutions at 25 °C using the Ubbelohde viscometer.

Prior to the preparation of solutions used for measurements of intrinsic viscosity, samples of both chitin and chitin diesters were dried under conditions of 60 °C for 3 h and kept in the desiccator.

2.2.1. IR spectra

IR measurements of samples of purified chitin and obtained chitin diesters were performed using an IR-Nexus FT-IR spectrometer (Thermo Nicolet). The IR spectrum of chitin sample was recorded by means of the KBr pellet method. IR spectra of DHCH and DBC samples were

Table 1
The results of DBC and DHCH synthesis under various conditions (time, temperature) versus reaction yield and final product intrinsic viscosity.

Reaction conditions		Chitin dibutyrate			Chitin dihexanoate		
Temp. [^{0}C]	Time [h]	Sample code	Reaction yield [%]	Intrinsic viscosity [dl/g]	Sample code	Reaction yield [%]	Intrinsic viscosity [dl/g]
0	2	54a	8.9	1.01	60a	70.34	2.15
	4	54b	21.1	1.43	60b	77.46	1.96
	6	54c	57.4	2.53	60c	87.02	1.57
	24	54d	71.8	1.85	60d	89.93	1.47
8	2	55a	18.1	1.33	62a	85.56	1.84
	4	55b	83.3	1.77	62b	86.63	1.75
	6	55c	83.7	1.63	62c	87.99	1.32
	24	55d	84.6	1.24	62d	88.65	0.70
21	2	56a	85.2	1.56	59a	84.52	1.14
	4	56b	82.1	1.32	59b	90.08	0.66
	6	56c	78.7	0.94	59c	91.14	0.54
	24	56d	70.4	0.45	59d	95.64	0.16