Reactive & Functional Polymers 73 (2013) 1639–1645

Contents lists available at ScienceDirect

Reactive & Functional Polymers

journal homepage: www.elsevier.com/locate/react

Influence of sulfated arabino- and glucuronoxylans charging-behavior regarding antithrombotic properties

Simona Strnad [a,*], Nena Velkova [b], Bodo Saake [c], Aleš Doliška [a], Matej Bračič [d], Lidija Fras Zemljič [a]

[a] University of Maribor, Faculty of Mechanical Engineering, Institute of Engineering Materials and Design, Smetanova ulica 17, 2000 Maribor, Slovenia
[b] TT Okroglica d.d., Dombrava 1, Volčja Draga, Slovenia
[c] University of Hamburg, Chemical Wood Technology, Department of Wood Science, Germany
[d] Savatech d.o.o., Škofjeloška c. 6, 4000 Kranj, Slovenia

ARTICLE INFO

Article history:
Received 26 August 2013
Received in revised form 26 September 2013
Accepted 27 September 2013
Available online 9 October 2013

Keywords:
Arabinoxylan
Antithrombotic properties
Charge
Glucuronoxylan
Sulfation

ABSTRACT

During the last decade xylans were widely researched and several derivatives have been developed and analyzed. However, the application properties of these new products are still sparsely known. The main objective of the presented investigation was to examine whether sulfated xylans from different sources (hard wood and oat-spelt) possess antithrombotic properties and how their protonation/deprotonation behavior in water solutions influence these properties. Two types of xylans, glucuronoxylan derived from beech wood, and arabinoxylan from oat spelt, were sulfated. Infrared spectroscopy, elemental analysis, NMR and size exclusion chromatography were applied for analyzing the success of derivatization. Deprotonation/protonation behavior of xylan samples in water solutions was analyzed by titration methods and the antithrombotic properties were investigated using activated partial thromboplastin time determination.

The results showed significant increases in negative charges for the sulfated samples, which were a consequence of introducing sulfated groups as strong acids. However, the increase of antithrombotic properties was not influenced only by the presence of certain amounts of sulfate functional groups but also by the total negative-charges originating from both sulfate and carboxyl groups. The later was proved by the high linear correlation between the activated partial thromboplastin time values, and the total charge of the samples.

1. Introduction

The development of haemocompatible biomaterials represents an extreme challenge, as cardiovascular complications are a major reason of deaths throughout the developed world. Heparin is currently the most widely used antithrombotic substance due to its excellent anticoagulant activity. However, it can cause certain adverse effects, like abnormal bleeding by the treated patients. Moreover, heparin is derived from mammalian sources and, as such, might be contaminated with animal proteins and pathogenic agents [1]. In order to avoid the risk of contamination from pathogenic agents, research today focuses on therapeutics derived from non-mammalian, mostly plant-sources [2]. Sulfated polysaccharides that are either of natural, semisynthetic or synthetic origin have displayed multiple biological activities; and their anticoagulant activity in particular has been widely studied [3,4]. It was discovered during previous research that the introduction of sulfate groups into polymer structures improves the antithrombotic properties of some polysaccharides [5,6]. Several methods for polysaccharide sulfation have been reported in order to introduce anticoagulant properties [7,8].

Xylans are hemicelluloses largely found in nature and are considered to be green polymers that may play an essential role in the renewability of waste products due to their biodegradable and biocompatible nature [9]. Furthermore their specific polysaccharide structures provide interesting platforms for different functionalization and derivatization in order to achieve water solubility, antioxidant activities or antiviral properties [10–14].

In last few decades, xylans had been investigated as renewable polymers for different applications, especially in nutrition [15,16], medical [17,18] and pharmaceutical [19] fields. Ebringerova and coworkers investigated mitogenic and comitogenic properties of xylans and compared it to the commercial immunomodulator zymosan [20]. Structurally and chemically modified xylans from birch wood were applied for nanoparticles preparation [21] and beech xylans were derivatised in order to prepare prodrugs for ibuprofen release [22].

* Corresponding author. Tel.: +386 2 220 7882.
E-mail addresses: simona.strnad@um.si (S. Strnad), nena.velkova@gmail.com (N. Velkova), bodo.saake@ti.bund.de (B. Saake), ales.doliska@um.si (A. Doliška), mattej.bracic@gmail.com (M. Bračič), lidija.fras@um.si (L.F. Zemljič).

1381-5148/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.reactfunctpolym.2013.09.007

Xylan sulfates are the only xylan derivatives, which have been continuously researched for their anticoagulant, antiviral and anticancer activities [23–25]. Recently a broad study was performed of sulfation procedures for xylans from different sources, and xylan sulfates with a broad structure variety were obtained and their structures analyzed [8]. However, there is still a gap in knowledge about relations between the chemical structures and specific application properties of such derivatized xylans, i.e. the relations between charging behavior and different aspects of biocompatibility.

The main aim of the presented investigation was to derivatize xylans in order to introduce sulfate groups as established by Martinichen-Herrero et al. [7]. Chemical characterization was performed by IR and NMR spectroscopy, elemental analysis, and size exclusion chromatography. Titration techniques and determination of activated partial thromboplastine-time (APTT) were performed in order to investigate the influence of charging-behavior on the antithrombotic properties of synthesized xylans. High linear correlation between the amount of deprotonated groups' content was pointed out together with antithrombogenicities of the derivatized xylans.

2. Experimental

2.1. Materials

Arabinoxylan, from oat spelts (OX) (Mw = 24,400 g/mol; Mw/Mn = 2.5) was obtained by sodium hydroxide extraction and precipitation as published previously [26]. 4-O-methyl glucuronoxylan from beech wood (BX) (Mw = 18,000 g/mol; Mw/Mn = 1.5) was obtained analogously by the extraction of beech wood holocellulose. The holocellulose was prepared by treatment of the starting material with 4.5% sodium chlorite for 120 h at room temperature [27].

The structural formulae of both xylans are represented in the Fig. 1. The 4-O-methylglucuronic acid (4-MGA) contents of the xylan samples (determined by 1H-NMR in NaOD) were for BX: 9.4 mol% and for OX: 3.8 mol% based on the anhydroxylose of the xylan backbone. The carbohydrate content in the xylan hydrolyzates was analyzed by borate-anion-exchange-chromatography with post-column derivatization and detection at 560 nm, as described previously [28]. The compositions regarding neutral carbohydrates of both samples are represented in Table 1. For BX the impurities of other carbohydrates add up to 3.8%. For the arabinoxylan from oat spelts 5% of other carbohydrates are included. The hydrolysis residue represents the residual lignin of the sample. For BX this is rather low (0.8%) while OX contains larger amounts of lignin impurities (5.5%). The mass balance of the analysis amounts only to about 80% for BX and 93% for OX. This is due to the fact that some components are not considered in the analysis e.g. ashes, extractives and acid soluble lignin.

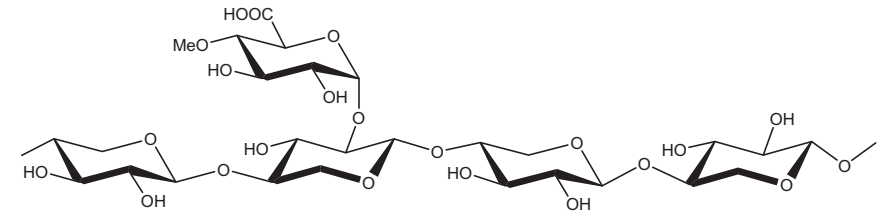
2.2. Methods

2.2.1. Sulfation

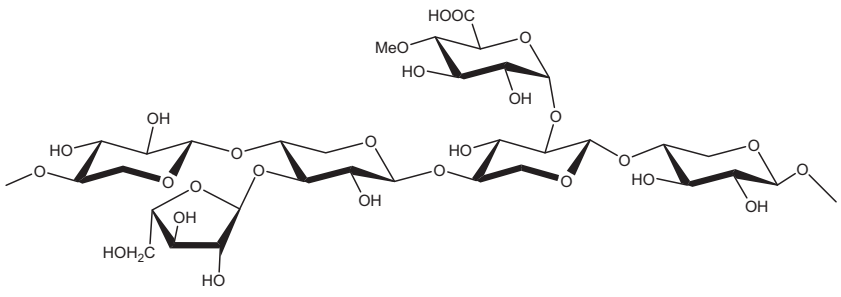
Both xylan samples were sulfated using chlorosulfonic acid according to the procedure of Martinichen-Herrero et al. [7]. In this study two different reaction times (5 h and 20 h) were applied in order to manipulate the amount of sulfate groups in xylan samples. 1 g of purified xylan sample was solubilized in a 15 mL of formamide (purity ~ 98%) and 10 mL of pyridine mixture, then vigorously stirred for 24 h followed by a drop-wise addition of 1.6 mL chlorosulfonic acid over 1 h in an ice-bath. The mixture was maintained for the next 4 h or 19 h, respectively, at room temperature. After each time ice–water was added followed by neutralization with 10% (w/v) aqueous $NaHCO_3$. The solution was dialyzed against deionized water for 3 days (the water was changed every 24 h) to remove pyridine, salts, and potential degradation products. Finally the solution was freeze-dried. The structural formula of the non-selectively sulfated xylan is represented in Fig. 2.

All the chemicals used were of analytical grade and used without further purification.

Denotations of samples are presented in Table 2.



BX



OX

Fig. 1. Chemical structures of 4-O-methyl-D-glucurono-D-xylan (BX) and L-arabino-4-O-methyl-D-glucurono-D-xylan (OX).