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Cationization of heparin for film applications

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ABSTRACT

Trimethylammonium-2-hydroxypropyl-(TMAHP) spacer was introduced into heparin (H) and the prepared films were characterized by elemental analysis, NMR, SEC-MALS, TG/DTG/DTA, AFM and mechanical tester. When quaternized at the ratio of H/NaOH/alkylating agent/H_2O = 0.1-1/0-2/0.1-1/50-500 mmol, H was substituted at A6 and A3 positions. The formation of double-substituted structures by substitution of free hydroxyl group of the previously introduced TMAHP substituent is evident. In the absence of NaOH (H/GTMAC/H_2O = 1:1:500) the most drastic decrease of M_n to 8.639 kg/mol and M_w/M_n at 1.48 was observed in comparison to H (M_n = 9532 g/mol with M_w/M_n = 1.38). The film mechanical properties were better on H (E = 4030 MPa; σ_b = 65 MPa; ε_b = 4.6%) than on quaternized specimens (E = 2500–3340 MPa; σ_b = 25–40 MPa; ε_b = 1.7–1.8%). The AFM images did not prove relation between mechanical properties and surface shape.

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1. Introduction

Macromolecular chemistry of heparin is mostly related to O- or N-deacetylation, O- or N-sulfation, or re-sulfation (Casu, Naggi, & Torri, 2002; Naggi et al., 1987; Yates et al., 1996; Mulloy, 2012; Shriver, Capila, Venkataraman, & Sasisekharan, 2012). Apart from desulfation, the enzymatic and chemical modifications under alkaline conditions can result to depolymerization, 2,3-epoxidation, and β-elimination (Ragazzi et al., 1993; Linhardt, 1992; Naggi et al., 2001). In addition, semi-synthetic derivatives were also prepared by N-acetylation, esterification, amidation, O-acylation or reductive amination (Fernández, Hattan, & Kerns, 2006). The introduction of TMAHP spacer under water-alkaline conditions was studied on xylan, including sulfated xylan, as potential analogues of heparin (Šimkovic, Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011a, 2011b). The comparison of xylan properties with those of heparin could open new perspectives for their applications and also utilize heparin for all-polysaccharide composite new products (Šimkovic, 2013). The goal of the present work is to learn about possibilities of heparin cationization with glycidyltrimethylammonium chloride (GTMAC), (S)-(−)-(3-chloro-2-hydroxypropyl)-trimethylammonium chloride (SCHPAC) or (R)-(+)-(3-chloro-2-hydroxypropyl)-trimethylammonium chloride (RCHPAC) in presence or absence of NaOH, optimize the reactions conditions, to characterize the possible products and learn about their properties. These reactions have not been studied on heparin yet (Prado & Matulewicz, 2014). It could be expected that by introduction of quaternary groups into heparin some new properties not known at the present could be found. The new properties might result due to interaction of quaternary groups with sulphates groups or prevent the interaction of the heparin groups with the surrounding environment. The possible applications might be in the medical field with properties analogical to already studied composites (Kaminski, Zazakowny, Szczubialka, & Nowakowska, 2008; Kemp & Linhardt, 2010).

2. Experimental

2.1. Materials

Heparin (Serva, # 24590.02), glycidyltrimethylammonium chloride (GTMAC; Aldrich, # 50053-50ML), (S)-(−)-(3-chloro-2-hydroxypropyl)-trimethylammonium chloride (SCHPAC; Aldrich # 329177-5G), (R)-(+)-(3-chloro-2-hydroxy-propyl)-trimethylammonium chloride (RCHPAC; Aldrich # 32,916-9) and all other chemicals were used without further purification.

2.2. Chemical modification

The mixtures at ratios heparin/NaOH/alkylating agent/H_2O = 0.1–1/0–2/0.1–1/50–500 mmol were reacted at 1250 rpm/room

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temperature for 24 h. The reaction was stopped by dilution of the mixture and dialyzed (Spectra/Por®; 1 kDa MWCO) until pH dropped to 7.20 and freeze-dried or casted on Petri dishes and dried at RT till constant weight. The amounts of reactants used, elemental compositions of products, the total degree of quaternization, sulfation, acetyl content, amount of free hydroxyl groups and water in the average dimer unit as well as yields are listed in Table 1.

2.3. Analytical methods

High-resolution NMR measurements of samples (~50 mg) were performed in D_2O (ARMAR Cat-No. 014400.0010) at 25 °C on Varian 600 MHz UNITY INOVA spectrometer equipped with 5 mm cryogenic probe. ^1H and ^{13}C chemical shifts were referenced to internal 3-(trimethylsilyl)-propionic acid (TSP). The pH-in-D_2O (pD) values of solutions were adjusted to values higher than 7.2 (Nguyen & Rabenstein, 2011).

The elemental compositions of specimens were performed on FLASH 2000 Organic elemental analyzer (Thermo Fisher Scientific; furnace temperature: 950 °C; PTFE column, 6 mm o.d./5 mm i.d. × 2 m; 65 °C; helium as carrier and reference gas flow: 140 and 100 ml/min, respectively; oxygen flow: 250 ml/min; 720 s run time; 12 s sampling delay; 5 s injection end).

The characterization of the molecular weight distribution (MWD) of samples was performed using a multi-angle laser light scattering (MALS) absolute detector on-line to a size exclusion chromatographic (SEC or GPC) system. A differential refractometer index (DRI) was used as concentration detector. An aqueous mobile phase (0.1 M CH_3COONH_4 at pH = 7.0) and two TSK gel PW_{XL} columns (G4000 and G3000 from Tosoh Bioscience) were used. The SEC-MALS experimental conditions were the following: 35 °C of temperature; 0.8 ml/min of flow rate; 100 μL of injection volume; about 3 mg/ml of sample concentration. The specific refractive index increment with respect to the mobile phase (d*n*/d*c*) was measured off-line by a Chromatix KMX-16 differential refractometer. The d*n*/d*c* values were 0.132 ml/g and 0.146 ml/g respectively for heparin and derivatives samples. The differences between d*n*/d*c* values of various derivatives were lower than the experimental uncertainty of the off-line measurement; consequently a constant value d*n*/d*c* = 0.146 ml/g was assumed for all derivatives. The "Recovered Mass" listed in Table 3 is the mass of sample eluting (i.e. recovered) from the SEC columns, expressed as % of the sample injected mass (sample concentration multiplied by injection volume). The recovered mass was calculated from the peak area of the DRI concentration detector after accurate calibration. All other analytical methods were described previously (Šimkovic et al., 2014).

Atom force microscopy (AFM) images in Fig. 3 were performed using BioScope Catalyst (Bruker, Santa Barbara, USA). Data were acquired using PeakForce QNM (Quantitative Nanomechanical Mapping) technique in air and evaluated by NanoScope Analysis 1.40 (Bruker). For this experiment samples were prepared by casting a 1–5% water solution on a disposable plate (WillCo Wells BV, Amsterdam, Netherlands). After evaporation of solvent (~24 h at RT) the plate with obtained film was placed into sample holder and AFM measurements were performed.

3. Results and discussion

3.1. Cationization

According to the elemental composition the average molar formula of heparin dimer unit (Fig. 1) could be calculated: $[C_{12}H_{13}O_8N(SO_3)_{2.5}Na_{3.5}(COCH_3)_{0.6}(OH)_{2.0}(H_2O)_5]_n$. The observed elemental analysis (C, 22.15; H, 3.68; N, 1.82; S, 11.14) is in agreement with theoretical values (C, 21.72; H, 3.67; N, 1.92; S,

Table 1
Reaction components (mmol), elemental composition and yields of products.

Sample/DQ[a]	H	NaOH	AA[b]	H_2O	Formula	Calculated/found [%]	Yield [%][c]
HQ1/0.30	0.1	–	0.1[d]	50	$[C_{12}H_{13}O_8N(SO_3)_{2.1}(Na)_{3.5}(OH)_{1.2}(CH_3CO)_{0.6}(C_6H_{14}ON)_{0.2}(C_6H_{15}ON)_{0.1}(HO^-)_{0.3}(H_2O)_{5.0}]_n$	C, 24.58/24.93; H, 4.25/4.24; N, 2.49/2.74; S, 9.18/9.41	93
HQ2/0.30	1	–	1[d]	500	$[C_{12}H_{13}O_8N(SO_3)_{2.1}(Na)_{3.4}(OH)_{1.7}(CH_3CO)_{0.6}(C_6H_{14}ON)_{0.2}(C_6H_{15}ON)_{0.1}(HO^-)_{0.3}(H_2O)_{4.0}]_n$	C, 25.28/25.53; H, 4.08/4.33; N, 2.56/3.05; S, 9.44/8.64	92
HQ3/1.10	1	2	1[d]	500	$[C_{12}H_{13}O_8N(SO_3)_{2.3}(Na)_{4.4}(OH)_{0.9}(CH_3CO)_{0.5}(C_6H_{14}ON)_{0.6}(C_6H_{15}ON)_{0.5}(HO^-)_{1.1}(H_2O)_{8.0}]_n$	C, 25.79/25.93; H, 5.21/4.55; N, 3.22/3.03; S, 8.07/8.84	85
HQ4/0.90	1	2	1[e]	500	$[C_{12}H_{13}O_8N(SO_3)_{2.4}(Na)_{4.3}(OH)_{0.9}(CH_3CO)_{0.4}(C_6H_{14}ON)_{0.5}(C_6H_{15}ON)_{0.4}(HO^-)_{0.9}(H_2O)_{8.0}]_n$	C, 24.54/24.62; H, 4.96/4.63; N, 2.99/2.83; S, 8.63/9.02	83
HQ5/4.00	0.1	2	1[e]	50	$[C_{12}H_{13}O_8N(SO_3)_{2.4}(Na)_{3.5}(CH_3CO)_{0.4}(C_6H_{14}ON)_{2.0}(C_6H_{15}ON)_{2.0}(HO^-)_{4.0}(H_2O)_8]_n$	C, 34.86/35.00; H, 7.28/6.76; N, 5.53/5.16; S, 6.06/6.79	79
HQ6/0.90	1	2	1[f]	500	$[C_{12}H_{13}O_8N(SO_3)_{2.3}(Na)_{3.9}(OH)_{1.1}(CH_3CO)_{0.6}(C_6H_{14}ON)_{0.5}(C_6H_{15}ON)_{0.4}(HO^-)_{0.9}(H_2O)_{9.0}]_n$	C, 25.27/25.59; H, 5.41/4.36; N, 3.01/2.95; S, 8.33/8.97	93

[a] Total degree of quaternization calculated from elemental analysis.
[b] Alkylating agent.
[c] (mmol of product according to calculated formula/mmol of heparin used according to calculated formula) × 100.
[d] GTMAC.
[e] (S)-(−)-(3-chloro-2-hydroxypropyl)-trimethylammonium chloride (SCHPAC).
[f] (R)-(+)-(3-chloro-2-hydroxy-propyl)-trimethylammonium chloride (RCHPAC).

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