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Solvent and oxygen effects on the free radical polymerization of 6-*O*-vinyladipoyl-D-glucopyranose

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Abstract

The vinyl ester-type glycomonomer 6-*O*-vinyladipoyl-D-glucopyranose was polymerized in water and alcohol solutions. In all cases, long polymerization times were necessary to achieve reasonable conversions. Depending on the nature of the solvent, polydisperse glycopolymers were obtained possessing a molecular weight ranging between 10,000 and 122,000 Da (PS equivalent). Higher alcohols appeared to act as chain transfer agents and oligomers with DP_n between 2 and 6 were indeed obtained when 2-propanol was the solvent. Also, thorough oxygen removal from the reaction mixture proved to be essential for the success of the experiment, plain nitrogen sparging being ineffective in most cases.

Keywords: Glycopolymer; Vinyl ester; Chain transfer

1. Introduction

Amid an increasing number of reports on the synthesis and the applications of glycopolymers, [1–4] little attention has been so far devoted to the preparation of poly(vinyl ester)-type glycopolymers. This lack of data persists in spite of the distinctive advantages that these materials may offer in terms of environmental biodegradability [5] and in vivo biocompatibility of the polymer backbone. Hydrolytic cleavage of the saccharide groups of a poly(vinyl ester)-type glycopolymer, leaves in fact a poly(vinyl alcohol) main chain; that is a material already used in a number of medical applications [6,7] and that does not seem to interact with cellular blood components [8]. Part of the reason for the small number of articles on the subject rests with carbohydrate-functionalized vinyl esters being more difficult to synthesise than their (meth)acrylic ester counterparts. Indeed, all reports on the preparation of vinyl ester-type glycomonomers focus on the enzyme-catalysed transesterification of a dicarboxylic acid divinyl ester with a carbohydrate [9–16]. Also, with respect to the polymerization reaction, vinyl esters are a class of relatively unreactive monomers that only polymerize via a radical mechanism and through unstable, unconjugated radical chain carriers whose propagation is very difficult to control.

Our centre has recently described the first synthesis of a well-defined poly(vinyl ester)-type glycopolymer [16] by controlling the radical polymerization of 6-*O*-vinyladipoyl-D-glucopyranose **2** (6-*O*-VAGlc) with suitable RAFT agents. In that study the unprotected glycomonomer was directly polymerized in methanol or water, thus eliminating two synthetic steps (protection and de-protection) from the overall glycopolymer preparation. Notably, this approach offers the advantage of avoiding the compositional variability observed in free glycopolymers obtained from a deprotection reaction [17]. The use of free carbohydrates though, imposes some solubility considerations. For

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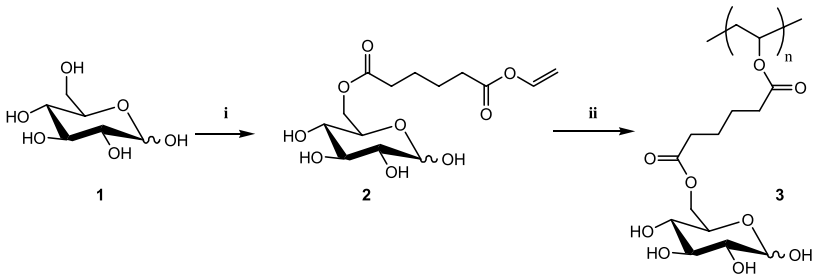


Scheme 1. Enzymatic synthesis of 6-*O*-vinyladipoyl-D-glucopyranose **2** and its free radical polymerization in protic media. Conditions: (i) Novozym 435, acetonitrile, 50 °C, 24 h; (ii) 4,4′-azobis(cyanopentanoic acid), 60–70 °C, 24–48 h.

instance, at room temperature 6-*O*-vinyladipoyl-D-glucopyranose **2** is only soluble in polar aprotic solvents such as pyridine, DMA, DMF and DMSO or in polar solvents capable of hydrogen bonding such as water and methanol. The free radical polymerization of 6-*O*-VAGlc has been previously investigated by Tokiwa and co-workers [18], who reported the formation of low molecular weight material when the monomer was reacted with azo-initiators in DMF (60 °C, 24 h; M_n ~4500 Da, PDI 1.9–2.3, SEC, PEO equivalent). Surprisingly, in a following paper from the same laboratory [19] a much higher molecular weight was reported for the same polymerization (M_n 34,400 Da, PDI 4.5, SEC, PS equivalent); possibly because of the use of a different molecular weight standard for SEC analysis. According to the authors, [18] polymerization in water with a redox initiator ($FeSO_4/H_2O_2$) resulted in the formation of an intractable gel as a consequence of the reaction between the pendant reducing sugar and hydrogen peroxide. The latter generates hydroxyl radicals on the formed polymer chains leading to branching and cross-linking.

As part of an optimization study on the free radical polymerization of vinyl ester-type glycomonomers, we now report the results for the polymerization of 6-*O*-vinyladipoyl-D-glucopyranose **2** (Scheme 1) in a number of protic solvents using two common techniques for oxygen removal: the sparging of nitrogen and consecutive cycles of freeze–evacuate–thaw.

2. Experimental

2.1. Materials and methods

Unless otherwise specified, all chemicals were reagent grade and used as received. 4,4′-azobis(cyanopentanoic acid) (98%, Fluka), carbon disulfide (99.9%, HPLC grade, Aldrich), deuterium oxide (99.9%, Cambridge Isotopes), ethanol (spectroscopic grade, Aldrich), ethyl acetate (99.5%, APS), methanol (99.7%, anhydrous, Aldrich) and 2-propanol (anhydrous, Aldrich) were used as received. Water was distilled prior to use. Accurate volumes were measured with an automatic pipettor (Eppendorf Research, 200–1000 μL) calibrated with distilled water (22 °C, $d_{H_2O} = 0.9878$, mean error = 0.05%) or with a gas tight syringe (50 μL). The synthesis of 6-*O*-vinyladipoyl-D-glucopyranose **2** (a white solid) was described elsewhere [16].

2.2. Analysis

NMR experiments were conducted on a Bruker Avance DMX300 spectrometer (resonance frequencies of 300.2 and 75.5 MHz for ^1H and ^{13}C nuclei, respectively). Molecular weights and molecular weight distributions were measured by size exclusion chromatography (SEC) on a Shimadzu modular LC system comprising a DGU-12A solvent degasser, a LC-10AT pump, a SIL-10AD auto injector, a CTO-10A column oven and a RID-10A refractive index detector. The system was equipped with a 50×7.8 mm guard column and four 300×7.8 mm linear columns (Phenomenex 500, 10^3, 10^4 and 10^5 Å pore size; 5 μm particle size). *N,N*-Dimethylacetamide (HPLC, 0.03% w/v LiBr, 0.05% BHT) was used as eluant at a flow rate of 1 mL min^{-1} while the columns temperature was maintained at 40 °C. Polymer solutions (3–5 mg mL^{-1}) were injected in 50 μL volumes. Calibration was performed with narrow polydispersity polystyrene standards (Polymer Laboratories) in the range 0.5–1000 kDa and SEC traces were elaborated with Cirrus 2.0 software (PL). ESI-MS analysis was performed with a Thermo Finnigan LCQ Deca ion-trap mass spectrometer (Thermo Finnigan, San José, CA) equipped with an atmospheric pressure-ionization source operated in nebulizer-assisted electro-spray mode (ESI). The instrument was calibrated with caffeine (Aldrich), MRFA (tetrapeptide, Thermo Finnigan), Ultramark 1621 (Lancaster) and polypropylene glycol (M_n 2700, Aldrich) in the mass range of 195–3822 amu. All spectra were acquired in positive ion mode over the *m/z* range 100–1000 or 500–4000 with a spray voltage of 5 kV, a capillary voltage of 35 V, a tube lens offset of −30 V and a capillary temperature of 275 °C. Nitrogen was used as sheath gas at a flow rate of 0.5 L min^{-1} and helium as the dumping gas.

2.3. Radical polymerization in water or methanol

In a typical experiment (runs 1 and 2 in Table 2), 6-*O*-

Table 1
Summary of radical polymerization experiments in water and different alcohols with oxygen removal via bubbling of nitrogen

Run no.	Solvent	Monomer (M)	Initiator (mM)	Temp. (°C)	React. time (h)	Conv. (%)[a]	M_n (Da) SEC[b]	M_w/M_n SEC
1	Water	0.28	0.27	60	24.0	34	59,300	1.53
2	Water	0.23	2.3	60	24.0	82	59,300	1.68
3	Methanol	0.27	0.27	60	32.8	0	–	–
4	Methanol	0.27	2.6	60	32.8	0	–	–
5	Ethanol	0.28	0.27	70	32.8	0	–	–
6	Ethanol	0.27	2.7	70	32.8	0	–	–
7	2-Propanol	0.29	0.27	70	32.8	0	–	–
8	2-Propanol	0.27	2.6	70	32.8	0	–	–

Degassing via nitrogen bubbling for 15 min.
[a] ^{1}H-NMR, calculated from the ratio between the peak area of the vinyl proton H-13 and the peaks area of the glucose ring hydrocarbon protons H-2 to H-6.
[b] Polystyrene equivalents.

vinyladipoyl-D-glucopyranose (1.12 g, 3.34×10^{-3} mol) was dissolved in water (12 mL) and part of the resulting solution divided to two Schlenk tubes (3.0 mL, 8.6×10^{-4} mol each). A calculated amount of 4,4′-azobis(cyanopentanoic acid) water solution (run 1: 1.75×10^{-2} M, 50 μL, 8.74×10^{-7} mol; run 2: 500 μL, 8.74×10^{-6} mol) was added to each tube and the same were sealed with a greased glass stopper and degassed by three freeze–evacuate–thaw cycles. The samples were then transferred to a water bath pre-heated at 60 °C and reacted for 24 h. At the end of the polymerization, they were removed from the bath, quenched in ice-water (5 min) and freeze-dried overnight. All samples were then analysed by ^{1}H-NMR and SEC for conversion and molecular weight determination (vide infra). Run 1: final conversion: 6%; M_n (SEC) 121,600; PDI 1.98. Run 2: final conversion: 71%; M_n (SEC) 122,500; PDI 2.38.

2.4. Radical polymerization in ethanol or 2-propanol

In a typical experiment (run 5 in Table 1), 6-*O*-vinyladipoyl-D-glucopyranose (425 mg, 1.18×10^{-3} mol), ethanol (3.0 mL) and a 4,4′-azobis(cyanopentanoic acid) ethanol solution (5.82×10^{-2} M, 1.26 mL, 7.34×10^{-5} mol) were added to a glass vial (8 mL). The vial was sealed with a rubber septum, oxygen was removed by nitrogen bubbling (30 min) and the sample was transferred to an oil bath pre-heated at 70 °C. After 48 h, the vial was removed from the bath, quenched in ice-water (5 min) and the solvent evaporated first under reduced pressure and then at the freeze-drier. After ^{1}H-NMR and SEC analysis, the remaining polymer was re-dissolved in water, precipitated in acetone and freeze-dried overnight. Final conversion: 63%. M_n (SEC) 15,500; PDI 1.69. ^{1}H-NMR (D_2O, 30 °C) δ (ppm): 5.21 (d, 0.46H, $J=3.6$ Hz, H-1α), 4.90 (br, CH chain), 4.64 (d, 0.54H, $J=7.9$ Hz, H-1β), 3.35–3.50 (H-2 to H-6), 2.47 (H-11 and H-8), 2.39 and 1.91 (br, H-14), 1.65 (br, H-9 and H-10). ^{13}H-NMR (D_2O, 30 °C) δ (ppm): 175.4 and 174.5 (C-12, C-7), 95.93 (C-1β), 92.02 (C-1α), 75.49 (C-3β), 73.95 (C-2β), 73.32 (C-5β), 72.54 (C-3α), 71.38 (C-2α), 69.65 (C-4 and CH chain), 69.08 (C-5α), 63.41 (C-6), 33.13 (C-8, C-11), 33.62 (C-14, CH_2 chain), 23.71 (C-9, C-10).

2.5. Conversion calculation

Conversions were estimated from the ratio between the peak area of the unreacted vinyl proton H-13 and the total peaks area of the glucose ring hydrocarbon protons H-2 to H-6. The following formula was used:

$$x = 1 - \frac{(A_{vinyl}/A_{Glc})_{poly}}{(A_{vinyl}/A_{Glc})_{mono}} \quad (1)$$

where A_{vinyl} and A_{Glc} are the peak area of the vinyl proton H-13 and the peaks area of the glucose ring hydrocarbon protons H-2 to H-6, respectively. Subscripts 'poly' and 'mono' refer to the polymerized sample and to the starting monomer, respectively.

3. Results and discussion

The radical polymerization of 6-*O*-VAGlc was initially studied in water and methanol. Due to the high reactivity of vinyl ester radicals, the use of these solvents was preferred because of their low chain transfer activity, because they can be obtained in high purity from commercial or in house sources and because they are stable over time. The investigation was then extended to the use of higher alcohols (i.e. ethanol and 2-propanol) in order to estimate the influence of chain transfer to solvent on the obtained molecular weights. For all experiments, 4,4′-azobis(cyanopentanoic acid) was chosen as the initiator since it dissolves both in water and in polar protic solvents.

Results from the free radical polymerization of 6-*O*-VAGlc **2** in water and alcohols are summarized in Table 1. The same initial concentration of monomer was used in all cases, while two different concentrations of initiator (~0.27 and 2.7 mM) were tested for each solvent. Although all polymerizations were run for at least 24 h at 60 or 70 °C, polymer was only obtained when water was the solvent (M_n

Table 2
Summary of radical polymerization experiments in water and different alcohols with oxygen removal via freeze–evacuate–thaw

Run no.	Solvent	Monomer (M)	Initiator (mM)	Temp. (°C)	React. time (h)	Conv. (%)[a]	M_n (Da) SEC[b]	M_w/M_n SEC
1	Water[c]	0.27	0.29	60	24.0	6	121,600	1.98
2	Water[c]	0.24	2.5	60	24.0	71	122,500	2.38
3	Methanol[c]	0.27	0.32	60	24.0	0	–	–
4	Methanol[c]	0.27	3.2	60	24.0	23	28,100	1.73
5	Ethanol[d]	0.28	17	70	48.0	63	15,500	1.69
6	2-Propanol[e]	0.27	0.32	70	24.0	0	–	–
7	2-Propanol[e]	0.25	3.0	70	24.0	18	10,700	1.16

[a] ^{1}H-NMR, calculated from the ratio between the peak area of the vinyl proton H-13 and the peaks area of the glucose ring hydrocarbon protons H-2 to H-6.
[b] Polystyrene equivalents.
[c] Degassing by: three cycles of freeze–evacuate–thaw.
[d] Degassing by: nitrogen bubbling for 30 min.
[e] Degassing by: two cycles of freeze–evacuate–thaw.

59,300, PDI ~1.6). A possible explanation may be that for this set of experiments, samples were de-oxygenated by bubbling nitrogen for 15 min through the solutions. This technique is effective in eliminating oxygen-induced inhibition and retardation phenomena with monomers such as styrene or methyl methacrylate. In the case of vinyl esters though, the high reactivity of the propagating radical makes chain transfer to oxygen a much more probable event [20] and a more effective degassing technique may be needed. For instance, in a recent investigation [21] we have found that the presence of oxygen induces a variable induction period and a slight retardation in the xanthate-mediated polymerization of vinyl acetate in bulk. For this reason, a set of experiments similar to those summarized in Table 1 was conducted in which repeated freeze–evacuate–thaw cycles were used to degas the polymerization solutions (Table 2).

In this latter case, the polymerization proceeded to some extent in all solvents, although a high concentration of initiator (17 mM) was used for ethanol and no reaction occurred when a sub-millimolar concentration (0.32 mM) of the same was used in methanol and 2-propanol. Also, the conversion achieved in the case of water was lower than in the previous set of experiments, whereas higher molecular weights were obtained (122,000 vs 59,000 Da). These last results are inconsistent with each other and suggest that the presence of a reducing sugar moiety in the monomer (and in the resulting polymer) might play a role. Tokiwa and Kitagawa [22] have already reported that at 25 °C the reducing sugar branches in poly(6-*O*-VAGlc) react with oxygen to produce superoxide and enediol radicals and that, in the presence of monomer, the latter can initiate a polymer chain [18,23]. Thus, if residual oxygen is present in solution during the polymerization of 6-*O*-VAGlc, it will generate an additional number of initiating radicals that will in turn produce branched polymers of lower molecular weight but in a higher yield.

The re-precipitated polymer from run 5 in Table 2 was used for NMR analysis and the resulting proton spectrum is shown in Fig. 1 (bottom). With respect to the spectrum of the starting monomer (top), the vinyl protons signals are no

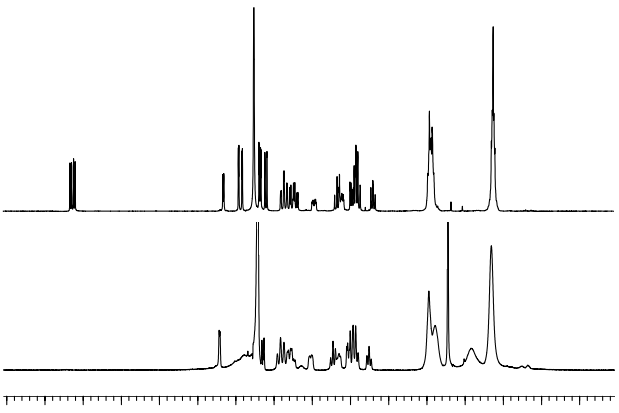


Fig. 1. ^{1}H-NMR spectrum (300 MHz, D_2O, 30 °C) of 6-*O*-vinyladipoyl-D-glucopyranose **2** (top) and poly(6-*O*-vinyladipoyl-D-glucopyranose) **3** (bottom) obtained from free radical polymerization in ethanol.

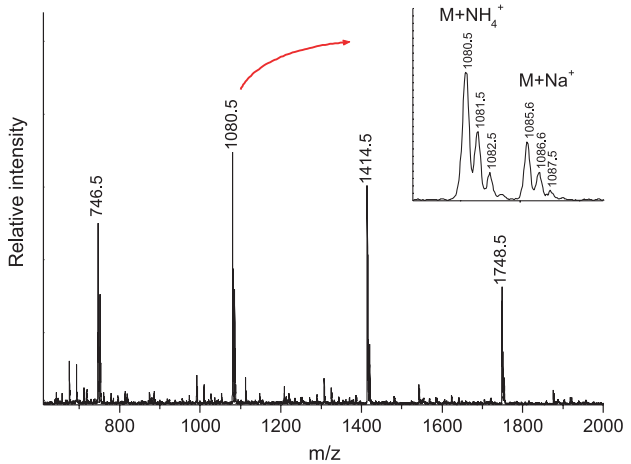
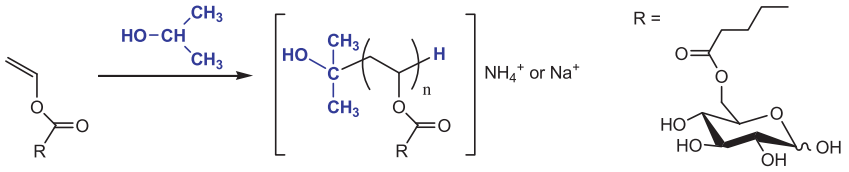


Fig. 2. ESI-MS spectrum of the oligomers obtained from the free radical polymerization of 6-*O*-vinyladipoyl-D-glucopyranose in 2-propanol (6-*O*-VAGlc 0.27 M, 4,4′-azobis(cyanopentanoic acid) 0.029 M, 80 °C, 22.3 h). Sample dissolved in acetonitrile–water 1:1 $NH_4^+HCO_2^-$ 1 mM (~5 mg mL^{-1}) for analysis. See Scheme 2 for peaks assignment.



Scheme 2. Structure of the oligomers produced by free radical polymerization of 6-*O*-vinyladipoyl-D-glucopyranose in 2-propanol and molecular ions observed by ESI-MS.

longer visible while three new broad peaks have appeared corresponding to the polymer chain methyne (4.90 ppm) and methylene (2.39 and 1.91 ppm) protons. The anomeric proton signals instead, are clearly distinguishable at 5.21 ppm (d, 0.46H, $J=3.6$ Hz, H-1α) and 4.64 ppm (d, 0.54H, $J=7.9$ Hz, H-1β) consistently with the expected polymer structure. ^{13}C-NMR data are also reported in the experimental part, confirming the structure of the polymer obtained.

Finally, average molecular weight of the obtained polymer decreased along the series water > methanol > ethanol > 2-propanol to indicate an increasing chain transfer to solvent reaction. In order to confirm this hypothesis, 6-*O*-VAGlc was polymerized in 2-propanol at 80 °C and the resulting polymer re-precipitated in acetone and injected to the electrospray ionization mass spectrometer (ESI-MS). Fig. 2 shows the obtained spectrum: a series of evenly spaced peaks was obtained 334 atomic units apart, a value closely matching the molecular weight of the monomer unit (334.2 Da). Moreover, the *m*/*z* value of all peaks matches that of ammonium or sodium adducts of 2-propanol-terminated oligo(6-*O*-VAGlc) chains, and confirms that the chain length obtained from polymerization in 2-propanol is completely determined by chain transfer to solvent reactions (e.g. *m*/*z* 746.5 exp. vs 746.3 calculated for $C_{31}H_{56}NO_{19}^{+}$, $n=2$; Scheme 2).

4. Conclusions

The vinyl ester-type glycomonomer 6-*O*-vinyladipoyl-D-glucopyranose was polymerized in water and alcohol solutions. In all cases, long polymerization times ($\geq$24 h) were necessary to achieve reasonable conversions. Depending on the nature of the solvent, a polydisperse glycopolymer was obtained having a molecular weight ranging between 10,000 and 122,000 Da (PS equivalent). Higher alcohols appeared to act as chain transfer agents and oligomers with DP_n between 2 and 6 were obtained when 2-propanol was the solvent. Finally, thorough oxygen removal from the reaction mixture proved to be critical for the success of the experiments, plain nitrogen sparging being ineffective in most cases. This might be due to the presence of a free reducing end in the sugar, and further investigations are in progress on the polymerization of non-reducing vinyl-ester type glycomonomers.

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