The influence of the chemical and structural features of xylan on the physical properties of its derived hydrogels

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Xylan polysacccharides, both with and without acetyl substituents, were obtained from the specific hardwood *Eucalyptus urograndis* by controlled extraction processes and eventually post-acetylated. They were subsequently functionalized with well-defined levels of methacrylic monomers to thereby provide different degrees of substitution of the functional group. These modified xylans were the basis to successfully prepare for the first time xylan/poly(2-hydroxyethyl methacrylate)-based hydrogels *via* the radical polymerization of HEMA, used as a crosslinking agent. The tuning of the crosslinking density of the hydrogel network was accomplished by preparing hydrogels that had two composition ratios of xylan to HEMA (60 : 40 and 40 : 60) and was also done by varying the degrees of substitution. The resulting hydrogels were characterized according to their morphology, swelling and rheological properties by field emission scanning electron microscopy (FE-SEM), gravimetric measurements after immersion in water, and dynamical mechanical analysis. Surprisingly, the presence of acetyl moieties leads to stiffer hydrogels which have a reduced capacity for water uptake. A natural extension to the synthesis and characterization of the novel-based xylan hydrogels is examining one of their primary functionalities: encapsulation and release. This functionality was one of the drivers of this work when it was conceived given the inherent ability of hydrogels to act as cargo carriers. Therefore, a representative anticancer drug doxorubicin was loaded into these hydrogels and its release in different media was studied. Acetylated xylans showed high delivery ratios while non-acetylated samples leveled off at half the level of the acetylated samples.

Introduction

Hydrogels are three-dimensional stable networks formed from cross-linked hydrophilic homopolymers or copolymers to form insoluble polymeric materials.[1,2] They have received increasing interest over the last several years in part due to their hydrophilicity, soft tissue-mimicking consistency, high permeability to metabolites and oxygen, and resilience. Furthermore, due to their unique biocompatibility, flexible methods of synthesis, range of constituents, and desirable physical characteristics, hydrogels constitute the basis for numerous biomedical applications. For example, they have been the material of choice for such applications as witnessed by work in cartilage or tendons, in bio-adhesives, in tissue engineering, as ocular lenses, or as drug delivery matrices.[3–5]

A wide range of synthetic polymers have been used to develop advanced hydrogels with tailored properties.[4] However, with the onset of the new "bio-"energy portfolio focused on extracting energy from biomaterials, the use of renewable resources has surfaced as a potential alternative to fossil fuels, and recent advances have streamlined the development of renewable biomaterials for advanced applications.[6]

In addition to their inherent renewability, natural polysaccharide-based hydrogels are currently attracting much interest for their fundamental properties such as tunable functionality, biocompatibility, and high degree of swelling.[6,7] Various polysaccharides have been investigated for hydrogel formulations, typically dextran,[8–10] alginate,[11–13] chitosan,[14–16] and starch.[17,18]

Heteropolysaccharides or hemicelluloses, although mostly unexplored as a raw feedstock for many polymeric material applications until recently, can nevertheless be as useful as related polysaccharides and have significant potential as a material resource for hydrogel preparation/application. For instance, xylan-based hydrogel has already shown potential for biocompatibility because of its reported non-cytotoxic effect.[19] However, there have only been few examples that have described the feasibility of using hemicelluloses such as galactoglucomannan to formulate hydrogels.[20–22]

Xylans are the most common hemicelluloses and considered to be the major non-cellulosic cell wall polysaccharide component of angiosperms (*e.g.*, trees, grasses, and cereals) where they exist in many different compositions and structures.[23] Indeed, xylans' structure exhibits a β-(1 → 4) linked D-xylosyl backbone, with various side groups or chains attached to the O-2 and/or O-3 of the xylosyl residues. These side chains mainly consist of α-D-glucuronic acid, 4-*O*-methyl-α-D-glucuronic acid and some neutral sugar units (α-L-arabinofuranose, α-D-xylopyranose or α-D-galactopyranose). Among the common side groups are also acetyl groups, phenolic, ferulic and coumaric acids.[24]

Relatively pure xylans and 4-*O*-methyl-α-D-glucuronoxylans have been used in various industrial and non-industrial applications including biomedical applications.[25] They have even been

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reported to inhibit the growth rate of tumors, probably with respect to the indirect stimulation of the nonspecific immunological host defense.[26,27] Xylan-based films were also reported to present a hydrogel-like behavior with high swelling capacity.[28,29]

The aim of this work is to fabricate, characterize, and explore hydrogels prepared from xylans extracted from hardwood as viable drug delivery vehicles. The study emphasizes on the effect of the acetyl groups by comparing xylans with different structural features, *e.g.*, the presence or absence of the acetyl groups. For hydrogel preparations, xylan backbones were conjugated with 2-hydroxyethyl methacrylate (HEMA) and the obtained hydrogels were characterized in terms of their morphology, swelling, and rheological properties. They were used as matrices to examine the potential for the delivery of an anticancer drug, *viz.*, doxorubicin, a representative anthracycline antibiotic and one of the most widely used anticancer drugs available, that has demonstrated high antitumor activity.[30]

Results and discussion

Extraction and derivatization of the xylans

First, acetylated and non-acetylated xylans were extracted from *Eucalyptus urograndis* using DMSO at 50 °C and 24% potassium hydroxide (w/v) at room temperature, respectively. As expected, the sugar composition showed an abundance of xylose concentration for both type of extractants with the presence of other minor monosaccharides such as glucose, mannose, rhamnose and galactose. In fact, the molar ratio of xylose to the other minor sugars detected was 0.83 : 0.17 for non-acetylated xylan and 0.89 : 0.1 for acetylated xylans which is well within the accepted range as found for the same xylans.[31,32] The respective structures of the isolated xylans were confirmed by NMR with representative recorded spectra in Fig. 1. The acetylated xylans had a degree of acetylation of approximately 0.63 as determined by ^{1}H-NMR which corroborated the values already published for this wood specie.[33] Similarly, the 4-*O*-methyl glucuronic acid (MeGlcA) contents were determined by ^{1}H-NMR from which it was found that these xylans had a ratio of 1.2 units of MeGlcA for every 10 xylose units. This value was approximately the same for both xylans.

The molecular weights of the extracted xylans were evaluated by Size Exclusion Chromatography (SEC). Acetylated xylans showed higher molecular weights in the range of 33.7 kDa, while the molecular weight of non-acetylated counterparts was 28.3 kDa. These results suggest that a slight degradation of alkali-extracted xylans due to β-elimination may have occurred during the extraction. The chromatography profiles also demonstrated a unimodal Gaussian distribution suggesting a relative structural homogeneity of isolated polysaccharides resulting from extensive purification based on solubilization–reprecipitation and dialysis which are known to separate small chains. This observation is supported by the low polydispersity indices that were approximately 1.06 for acetylated xylans and 1.33 for non-acetylated xylans.

In general, hydrogels as a class of polymeric-based materials represent a compositional motif best characterized as a 3D network and their preparation thus requires a cross-linking of polymer chains. To this end, the extracted xylans were first

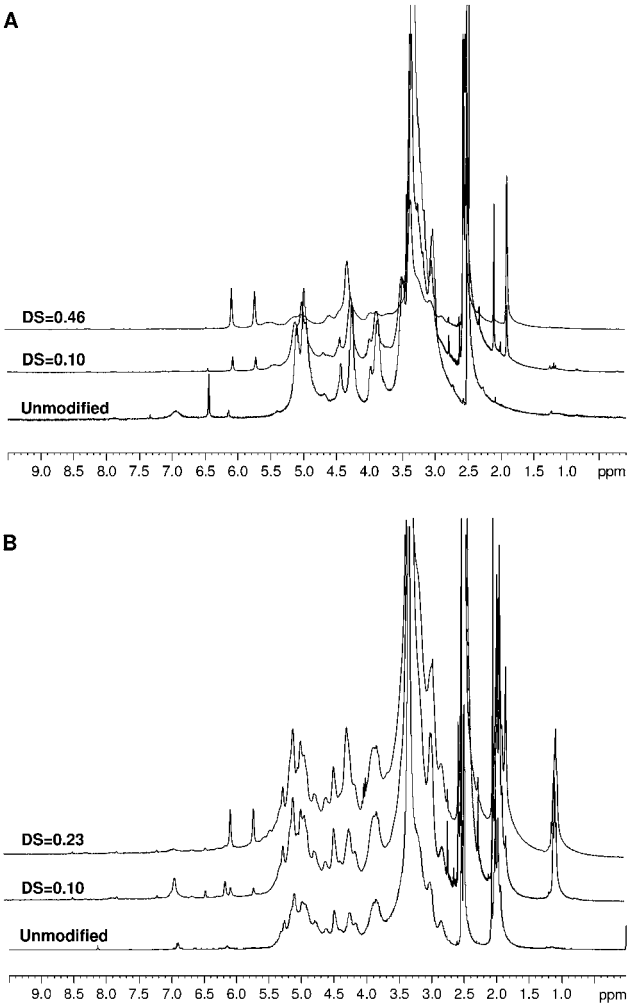
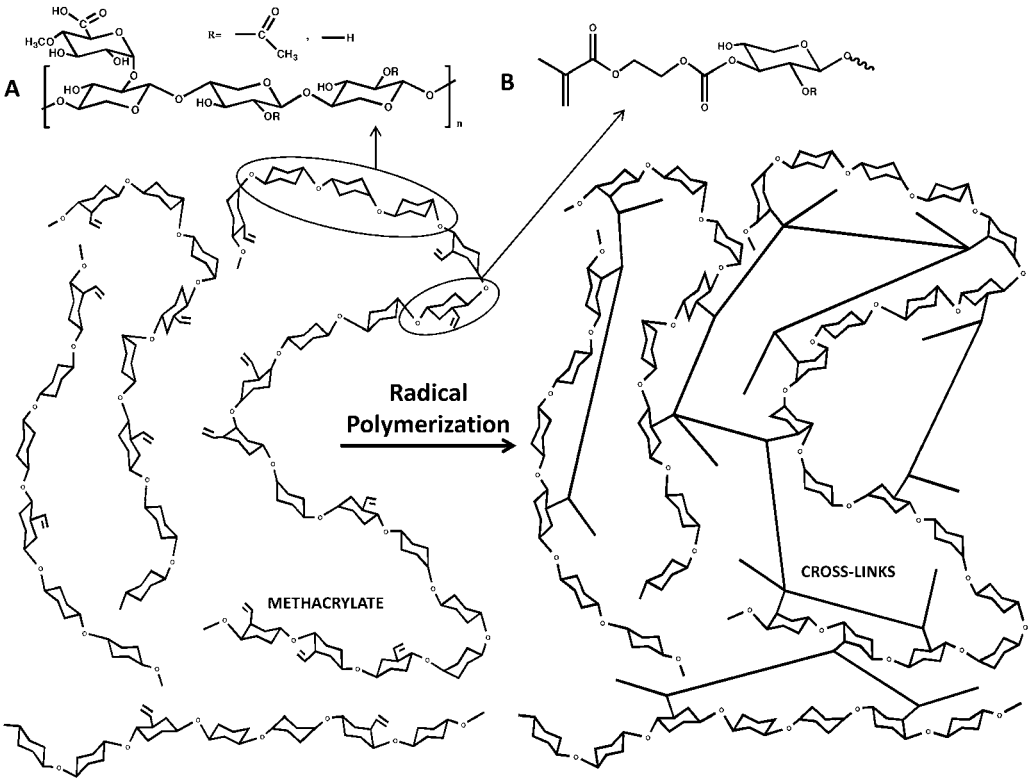


Fig. 1 ^{1}H-NMR spectra of unmodified and HEMA-Im-modified non-acetylated (A) and acetylated xylans (B).

covalently modified with 2-[(1-imidazolyl)formyloxy]ethyl methacrylate (HEMA-Im)[34] to produce hydroxyethyl methacrylate derivatized xylan (xylan-HEMA) (Scheme 1). This attached monomer further serves as a reactive site to crosslink the chains during the radical polymerization. By increasing the amount of attached HEMA-Im in the xylans' backbone, *i.e.*, the degree of substitution, an increase in the junction points (crosslinking density) is expected.

The HEMA-Im, previously prepared similarly to what has been reported, was coupled to the hydroxyl groups of xylans. The reaction time was varied from 6 to 120 h to achieve different degrees of substitution (DS) of HEMA on xylan backbone that allows for tuning the density of the 3D network. The success of this reaction was confirmed by ^{1}H-NMR and examples of recorded spectra are in Fig. 1. The attachment of HEMA moieties was evidenced by the presence of the signals at chemical shifts δ 6.05 and δ 5.71 ppm that are attributed to the vinyl proton of HEMA-Im.

The DS was determined by relating the intensity of the HEMA-Im vinyl proton signals to the intensity of signals of the acetyl groups for acetylated xylan or 4-*O*-methyl glucuronic acid for non-acetylated xylan, respectively. Fig. 2 illustrates the



Scheme 1 Representation of the xylan-based hydrogels formation by radical polymerization of the methacrylate groups. Chemical structures of (A) xylan polymer and (B) xylan–HEMA.

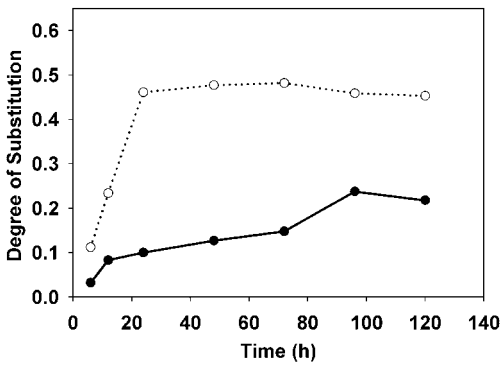


Fig. 2 Variation of the degree of substitution for non-acetylated (○) and acetylated (●) xylans modified with HEMA-Im as a function of reaction time.

variation of DS with reaction time. The results clearly indicate that the resulting DS varied from 0.02 to 0.23 for acetylated xylans and from approximately 0.10 to 0.46 for non-acetylated samples over reaction times from 6 h to 120 h. The higher DS (~0.46) achieved for non-acetylated xylan when compared to the acetylated ones is most likely due to the presence of more available hydroxyl groups because of the absence of acetyl groups. The presence of acetyl groups in acetylated xylans may have prevented the reaction of their vicinal hydroxyl groups due to steric hindrance (this factor can limit the diffusion of HEMA-Im to hydroxyl groups). This factor again may have affected also the rate of the reaction which can be easily recognized in Fig. 2. In the case of non-acetylated xylans the maximum DS (~0.46) was reached after approximately 24 h and longer times did not lead to any substantial further increases in the DS;[34,36] while the maximum DS achieved for the acetylated samples (*e.g.*, 0.23) was achieved 72 h later.

Morphology, bounded water and rheological properties

These modified xylans were used to manufacture the hydrogels, and HEMA was used as a co-monomer to accomplish the cross-linking between the xylan chains *via* a radical polymerization. Hydrogels with two compositions, namely 40 : 60 and 60 : 40 of xylans : HEMA were prepared and xylans with different DS were used for each type of xylan; however, thereafter, to accentuate the remarkable activity of these materials, the focus of the results obtained will be on the two DS, *i.e.*, low and high as shown in Table 1.

The amount of bounded or associated water on swollen hydrogels is demonstrated in Table 1. The uptake water molecules first disrupt the intermolecular hydrogen bonds and then hydrate/bind the most polar, hydrophilic sites. These molecules are distributed homogeneously throughout the polymer.[37]

The results showed that hydrogels produced with non-acetylated xylans had a higher bounded water content than those made from acetylated xylans. The absence of acetyl groups confers the structure greater hydrophilicity and therefore, a higher concentration of water molecules is able to bind to the polymer. Moreover, when the ratio of xylan : HEMA is increased from 40 : 60 to 60 : 40, it is possible to observe an enhancement in the bounded water in the hydrogels and this

Table 1 The composition and content of the bounded water of hydrogel-based xylans

Xylan type	Degree of substitution	Ratio xylan : HEMA	Bounded water (%)
Acetylated	0.10	60 : 40	51
Acetylated	0.23	60 : 40	59
Acetylated	0.10	40 : 60	50
Acetylated	0.23	40 : 60	55
Non-acetylated	0.10	60 : 40	66
Non-acetylated	0.46	60 : 40	72
Non-acetylated	0.10	40 : 60	56
Non-acetylated	0.46	40 : 60	64

behavior is more striking in the non-acetylated xylan, in accordance with the level of polymer used.

The resulting hydrogels were examined in terms of their morphology, swelling and deswelling behaviors, and rheological properties. The hydrogels present different macroscopic morphologies; the gels made from non-acetylated xylan were soft, whereas the ones made from the acetylated xylans were harder to solid-like gels. Fig. 3 shows typical SEM micrographs for the resulting gels. All formulations provided gels with porous structures where the size of the pores seems to be affected by both the type of xylans and their DS which provides a semi-quantitative way to ascertain the density junction points in the network. Open structures with larger pores were obtained with non-acetylated xylans which is in contrast to the case of acetylated samples that formed hydrogels with smaller pores. Furthermore, the structure became densely packed because the pores become smaller when the degree of substitution increased in both xylans. This fact was more pronounced in the case of highly substituted acetylated xylan-based hydrogels that showed a more firm morphology. This observation could be related to the presence of acetyl groups that confer to them a more "hydrophobic" character and therefore lead to enhanced affinity.

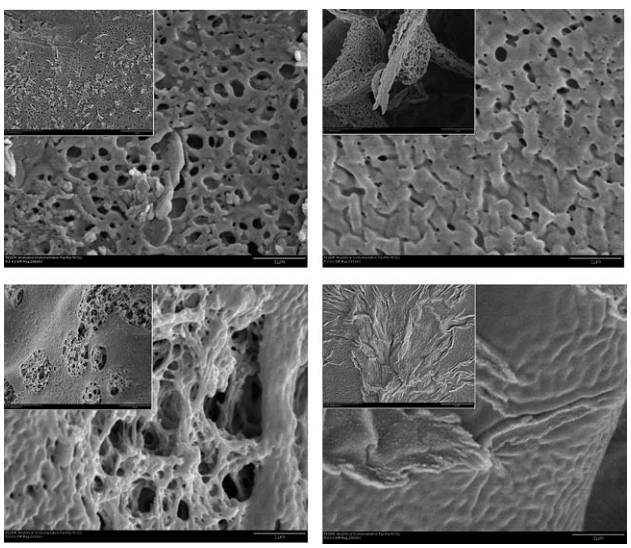


Fig. 3 SEM images of xylan based hydrogels with xylan to HEMA of 60 : 40 (top: non-acetylated xylans (left DS of 0.10 and right 0.46) and bottom: acetylated xylans (left DS of 0.10 and right 0.23)).

The rheological properties are strongly related to the number of effective intermolecular cross-links formed in the hydrogels as has been suggested from previous studies.[38–40] Hence, the mechanical behavior of the gel depends mainly on the architecture of the polymer network. Frequency sweep tests are typically used to evaluate the stability of 3D cross-linked networks.[41] The rheological properties of the resulting hydrogels were studied for all different compositions. The measurements were done on the hydrogels as taken from the reaction media, and the relative water content was around 80% for all samples.

Fig. 4 shows the rheological properties (storage moduli, G', and loss moduli, G'' and viscosity) of hydrogels based on acetylated or non-acetylated xylans having different DS as a function of the applied oscillating frequency. G' and G'' of all hydrogels obtained from non-acetylated xylans exhibited a frequency-independent behavior at very low frequencies (in the range of 0.01–2 Hz for low DS and 0.01–1 Hz for high DS) which is indicative of a stable, cross-linked network. However, at higher frequencies, all hydrogels showed an increase in G' and G'', and this behavior is more striking in the case of high DS and/or low xylans content (40%). Moreover, higher cross-linking density, resulting from higher DS or lower xylans content (because the same amount of the co-monomer was used), contributes to a sharp increase in the modulus. In fact, at higher frequencies, polymer chains, in highly cross-linked networks, fail to rearrange themselves rapidly upon the imposed stress and therefore stiffen up. Similarly, the change of the viscosity upon the applied stress demonstrated the same trend. The rheological properties of the resulting hydrogels seem to be also greatly influenced by the presence of the acetyl groups. Indeed, a significant increase in the stiffness of the hydrogels was observed because the storage modulus increased more than 1000-fold from approximately 0.6 MPa to more than 390 MPa in the case of hydrogels made with a high amount (60%) of highly substituted xylans (0.46 for non-acetylated *vs.* 0.23 for acetylated). For all samples, the storage shear modulus G' is higher than the loss shear modulus G'' over the entire frequency range which indicates that the elastic response of the material is stronger than the viscous response. In addition, the elastic modulus of the acetylated xylan based hydrogels exhibited a predominantly frequency-independent profile, especially those made from highly substituted xylans, throughout the range of applied frequencies which confirm their solid-like gel structures. Indeed, similar results were reported with hydrogels based on acetylated galactoglucomannan extracted from softwood (*Picea abies*).[42] Acetylated xylan based hydrogels are mildly cross-linked networks with a large free volume that allows them to respond nearly instantaneously and reversibly to external stresses with a rapid rearrangement of the polymer segments. When comparing the same DS and the same HEMA content, gels made with acetylated xylans appear to be more stiff and their G' demonstrates a frequency independence compared to those made with non-acetylated xylans that convincingly demonstrates their mildly cross-liked architecture even at the same DS.

This peculiar behavior is most likely related to the occurrence of the entanglement of xylan chains when acetyl groups are absent. Quite possible there exists a contribution of water molecules to such an entanglement-driven gelation in the case of non-acetylated xylans. Indeed, it is well known that through

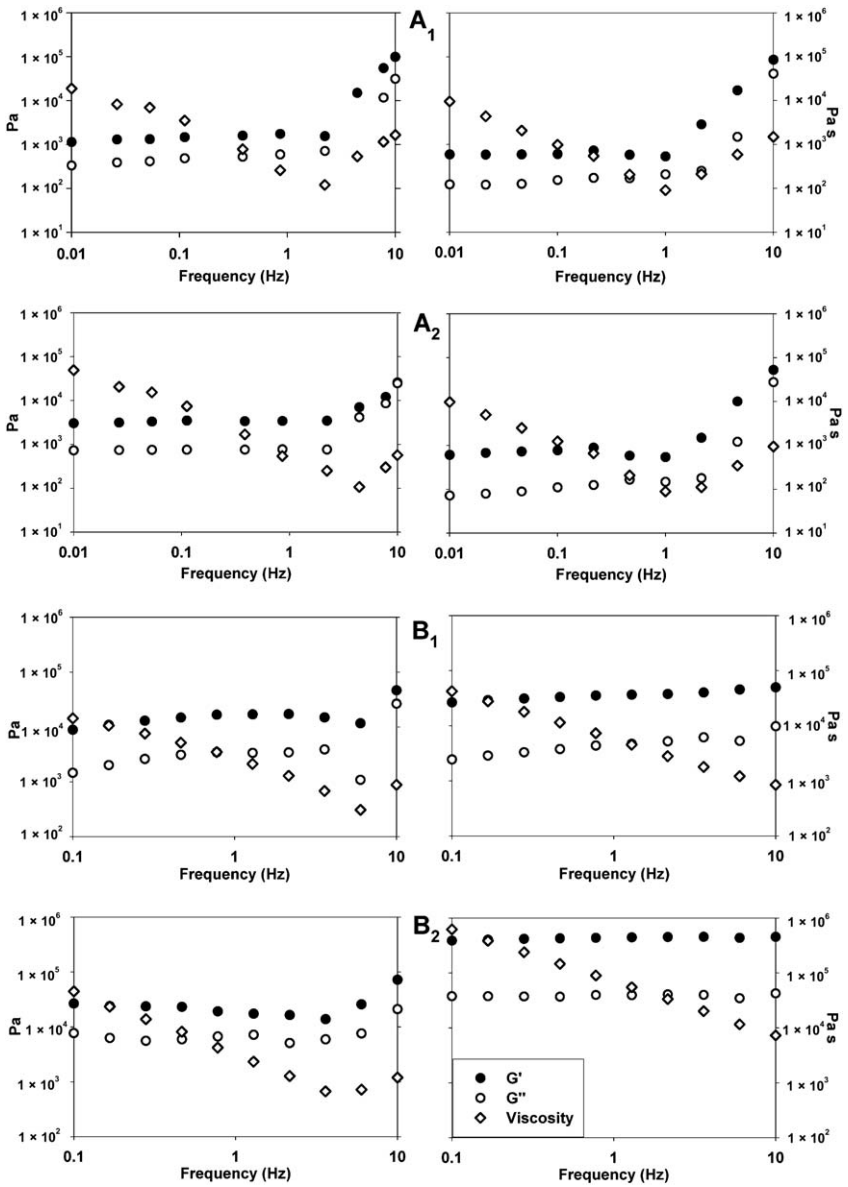


Fig. 4 Rheological behavior of hydrogels based on (A_i) non-acetylated (left DS = 0.10 and right DS = 0.46) and (B_i) acetylated (left DS = 0.10 and right DS = 0.23) xylans with different xylan : HEMA ratios (i = 1, 40 : 60 and i = 2, 60 : 40).

hydrogen bonding water molecules can be absorbed to xylan chains. These water molecules induce self-assembling of the backbone of xylan in a three-fold, left-handed conformation.[43,44] Such helix forming phenomenon is well described in several other polysaccharides like β-(1 → 3)-linked D-xylans,[45] and β-(1 → 3)-linked D-glucans.[46–48] In the case of xylan-based hydrogels, the presence of bonded water molecules may contribute to the cross-linking which "indirectly" increases the density of network junction points in the case of non-acetylated xylan based hydrogels. The presence of acetyl groups in the case of acetylated counterparts prevents such absorption. The amount of bounded water was in fact estimated by DSC measurements and as reported in Table 1, the total content of non-freezable bound water (bounded to HEMA copolymer and xylan backbone), is slightly higher in the case of non-acetylated xylans for hydrogels prepared under the same conditions (DS and HEMA content) which to a great extent confirms the earlier hypothesis.

Swelling behavior

The cross-linking density of the network seems to also affect the water uptake (swelling) behavior of the hydrogels; a less dense network confers more free volume to hydrogels and consequently the ability to absorb more water. The swelling behavior of a hydrogel is an important parameter for drug release because it determines the optimal concentration of the drug loading. However, it has been conjectured that the conjugation of HEMA to the polysaccharides *via* the formation of ester bonds may be subject to hydrolytic cleavage that affects (i) the swelling of the gel,[9] (ii) the release of entrapped drugs, and (iii) depending on the extent of the hydrolysis of the ester bonds, it may result in dissolution of the gel.[35] To check the stability of the hydrogels with respect to the dissolution caused by hydrolysis of esters bounds,[35] the gels were immersed in water at 37 °C for 15 days. The swelling ratio was recorded from 0.25 hours to 24 hours of

immersion in water at 37 °C. As expected for polymethacrylate ester hydrogels, the hydrogels produced were very stable and no dissolution was observed in any of the hydrogels, as expected according to previous work.[9] Fig. 5 shows the results of the swelling behavior of all hydrogels produced from different types of xylans. These results demonstrate that all gels swell, by immersion in water medium, very quickly as they reach equilibrium after approximately only 1 hour. However, the extent of the swelling seems to be greatly affected by the type and DS of xylans and consequently the density of the hydrogel network. As expected, non-acetylated xylan-based hydrogels were able to uptake more water than their acetylated counterparts as evidenced by the swelling ratio (Q_S) reaching up to 4-fold. In fact, the presence of the acetyl groups confers a hydrophobic character to the hydrogels and consequently prevents water absorption. The swelling ratio obtained for the acetylated xylan is comparable to what has been reported for the acetylated galactoglucomanan. Conversely, the DS of the xylan does not appear to affect strongly the swelling behavior of hydrogels made from acetylated xylans. However, hydrogels made with low DS exhibit higher water swelling in the case of non-acetylated xylans. These findings reflect that a decrease density of the hydrogel network resulting from low DS leads to gels with large free volumes which enable them to absorb more water if their hydrophilic–hydrophobic balance is not severely compromised by the presence of substituents such as acetyl groups.

Post-acetylated xylan based hydrogels

In order to confirm the effect of the acetyl groups on hydrogel properties, non-acetylated xylans, previously extracted with alkali solution, were post-acetylated with acetic anhydride in the presence of pyridine. The success of the acetylation was confirmed by ^{1}H-NMR, as shown in Fig. 6B, and the achieved degree of acetylation was of 0.38 as determined by NMR.

Hydrogels were produced from post-acetylated xylans by following the same route previously described. They were first modified by HEMA monomer and the success of this grafting was also confirmed by NMR (see Fig. 6C). The modification of xylan with HEMA-Im was carried out to achieve the same DS_{HEMA} of 0.10. Hydrogels were further prepared based on the previously modified post-acetylated xylans and only the hydrogel

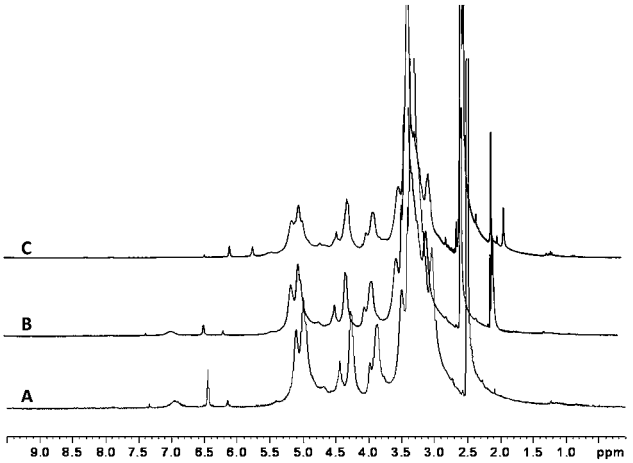


Fig. 6 ^{1}H-NMR spectra of (A) unmodified, (B) post-acetylated and (C) HEMA-Im-modified non-acetylated xylans.

composition xylan : HEMA ratio 60 : 40 was studied. The hydrogels made from the post-acetylated xylans present a macroscopic consistency similar to those prepared from acetylated xylans. Although the degree of acetylation obtained by the post-reaction is less than for naturally acetylated xylans, rheological measurements reported in Fig. 7A showed clearly a behavior unlike what has been previously seen in the case of hydrogels based on naturally acetylated xylans (Fig. 4B2, left). In fact the G' and G'' exhibited a predominantly frequency independent behavior in addition to a great increase of the stiffness ($G' = 16.9$ MPa) compared to hydrogels based on non-acetylated xylans ($G' = 3.1$ MPa). Likewise, the swelling behavior of hydrogels based on post-acetylated xylans is similar to the naturally acetylated xylan based hydrogels as the water uptake leveled off below $Q_S = 2$ compared to more than $Q_S = 4$ obtained in the case of non-acetylated xylans based hydrogels. These results confirm clearly the effect of acetyl groups of the physical properties of the xylan based hydrogels.

Drug release

The release of doxorubicin from different xylan-based hydrogels was investigated under simulated pH that closely approximated

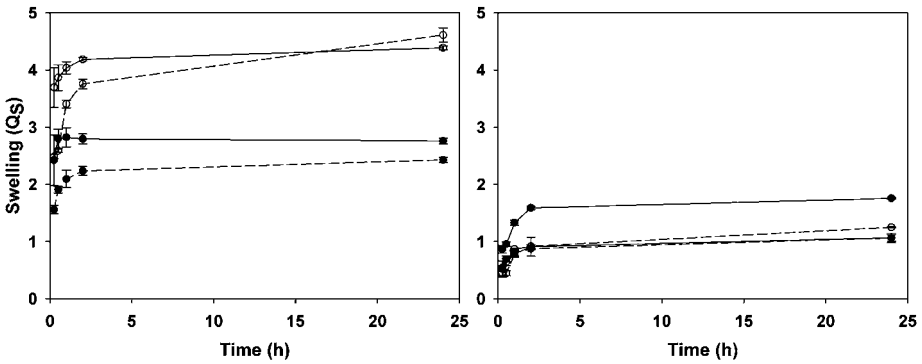


Fig. 5 Variation of swelling ratios *versus* time of hydrogels based on non-acetylated (left) and acetylated (right) xylans (solid line 40 : 60 and dashed line 60 : 40 of xylan : HEMA and (○) DS 0.1 and (●) DS 0.46 for non-acetylated or 0.23 for acetylated xylan, respectively) (average ± SD, $n = 2$).

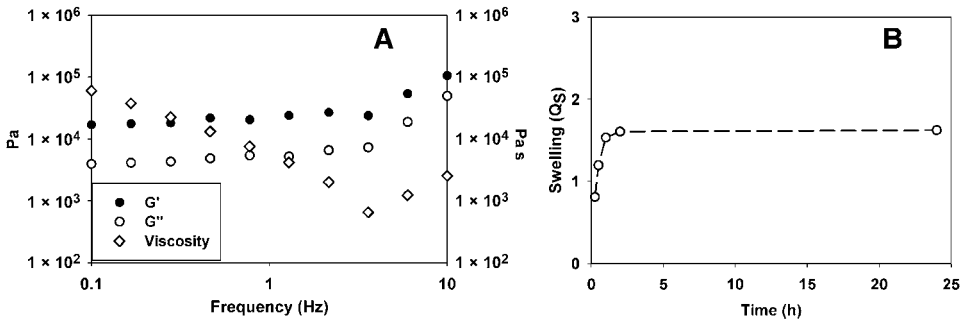


Fig. 7 A) Rheological behavior and (B) variation of swelling ratio *versus* time of hydrogels based on post-acetylated xylans (DS_{HEMA} = 0.10 and xylan : HEMA ratio 60 : 40).

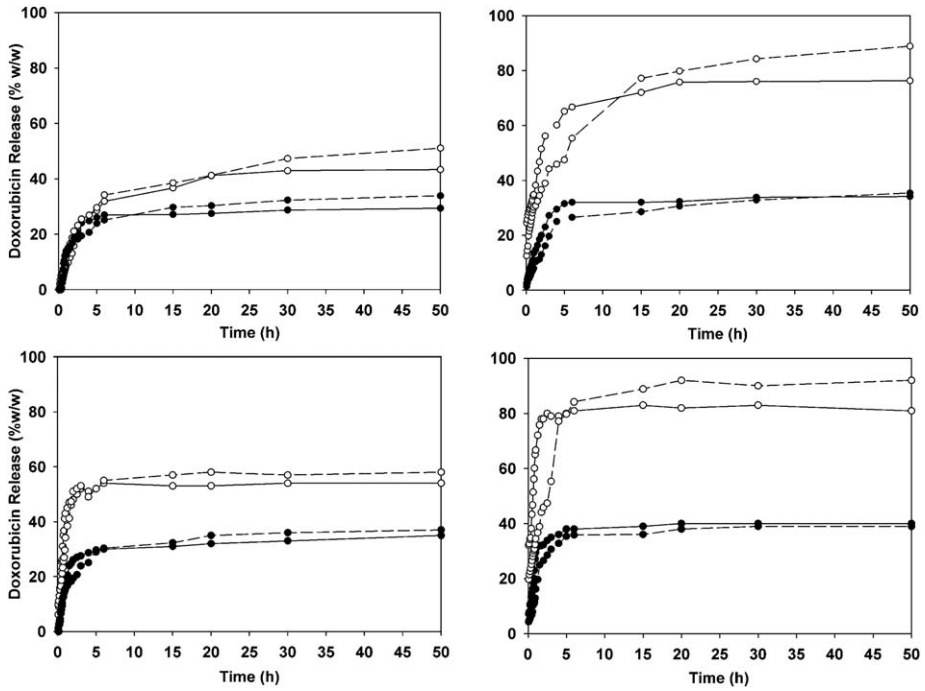


Fig. 8 Doxorubicin release from non-acetylated xylan (left) and acetylated xylan (right) based hydrogels as a function of time in different pHs (top pH 7 and bottom pH 2.5) (solid line 40 : 60 and dashed line 60 : 40 of xylan : HEMA and (○) DS 0.1 and (●) DS 0.46 for non-acetylated or 0.23 for acetylated xylan, respectively).

the gastrointestinal tract environment. Thus, two representative pH were used, namely 7.0 and 2.5 for the release study and the results are depicted in Fig. 8.

The two types of hydrogels showed different profiles as hydrogels made from non-acetylated xylans leveled off between 30 and 60% depending on the DS and xylan content used. This behavior is directly opposed to what was observed for hydrogels made from acetylated xylans in which more than 80% of drug was released for high DS and high xylan content. This effect may be attributed to the diffusion of the drug caused by rapid gel swelling and also the release of drug that is adsorbed towards the surface of the gel matrix.[49] This drug adsorption could be reduced by the presence of acetyl groups which enhance its delivery from hydrogels derived from acetylated xylans. The higher and faster release of doxorubicin in low DS hydrogels may also be explained because of the presence of larger pores, observed by SEM, especially in acetylated hydrogels.[50]

The ratio of doxorubicin released was not affected by changing the pH; however, the release rate from all hydrogels was faster at lower pH and two reasons for this behavior could be posited: (1) less stability of xylans at lower pH and (2) protonation of the hydroxyl groups of the xylan at lower pH accelerates the swelling, consequently accelerating the release of doxorubicin. Similar behavior was reported in hydrogels based on polyethylene glycol (PEG).[51]

Conclusion

In this work, not only was the success of preparing hydrogels from xylan, the most abundant non-cellulosic-based polysaccharide found in the plant kingdom, demonstrated, but also the overall richness/tunability of the delivery system based on the chemical functionality of the substrate. This renewable resource was extracted from *E. urograndis* by two different processes that

ultimately provided xylans with and without acetyl moieties. Subsequently, xylan/poly(2-hydroxyethyl methacrylate)-based hydrogels were prepared after facile crosslinking induced by methacrylic monomers using standard radical polymerization. Herein is demonstrated for the first time the effect of xylan-based acetyl substituents on the morphology and physical properties of a novel polysaccharide-based hydrogel biomaterial which was also confirmed by the post-acetylation of non-acetylated xylans. Most importantly, the presence of acetyl groups introduced compactness and high stiffness to the hydrogels which ultimately reduced their water swelling capacity and moreover, significantly enhanced their drug release properties as evidenced by the time release profile obtained for a representative drug, *i.e.*, doxorubicin.

Material and methods

Materials

Doxorubicin hydrochloride (99%) was purchased from Tocris Bioscience, *N*,*N*′-carbonyldiimidazole (CDI, 97%), 2-hydroxyethyl methacrylate (HEMA, 99%), triethylamine (TEA, 99.5%), ammonium peroxodisulfate (98%), sodium metabisulfite (98%), sodium sulfate (99%), potassium hydroxide (99%), hydrogen peroxide (30%) were all obtained from Sigma-Aldrich. Acetic acid (99.5%) and formic acid (99%) were purchased from Acros Organics. All reagents were used as received. Organic solvents (dimethyl sulfoxide (DMSO, 99.5%), ethyl acetate (99%), anhydrous chloroform, ethanol (99%), methanol (99%) and acetone (99.9%)) were all obtained from Sigma-Aldrich and used as received without any further purification.

Isolation of xylans

The xylans used in this work were extracted from hardwood *E. urograndis* specimens. First, extractive-free sawdust of wood samples were subjected to delignification with peracetic acid solution (pH 4.5) at 15%, as previously published,[52] to yield so-called holocellulose. Acetylated xylans were solvent-extracted from the holocellulose by DMSO (1 : 25 (w/v) holocellulose-to-DMSO ratio) at 50 °C, for 12 h, with stirring under nitrogen atmosphere. The DMSO dissolved xylans were acidified with formic acid to pH 2 and precipitated with an excess of ethanol. The xylans were then recovered by centrifugation, washed extensively with methanol to remove DMSO and dried at room temperature under vacuum. Non-acetylated xylans were alkali-extracted from the holocellulose by using KOH solution (24%) at room temperature, for 24 h, with stirring under nitrogen atmosphere. After acidification to pH 2, the extracted xylans were recovered as previously described.

Xylan acetylation

Acetylation of the xylan extracted with alkali was carried out with pyridine : acetic anhydride (1 : 1) at 25 °C for 48 hours.[53] The acetylated xylans were recovered by evaporation of the acetylation mixture several times (pyridine/Ac_2O) with ethanol.

Sugar analyses

Sugar compositions of the extracted xylans were determined by high performance liquid chromatography (HPLC) analysis after acid hydrolysis of the polymers. Xylans were first pre-hydrolyzed by 72% (w/v) sulfuric acid at room temperature for 2 h followed by sulfuric acid (4% w/v) catalyzed hydrolysis at 120 °C for 1 h.[54] The resulting solutions were diluted with ultra-pure water to the desired concentration and filtered through a 0.2 μm nylon filter (Millipore, Billerica, MA) before chromatography analysis. They were then analyzed by using a Dionex ICS 3000 IC system equipped with a CarboPac PA1 cartridge, an eluent generator (EG50) and an electrochemical detector (ED50). The mobile phase was 18 mM hydroxide (OH–) and all analyses were conducted with a column temperature of 25 °C.

Size Exclusion Chromatography (SEC)

The isolated xylans were dissolved in a small amount of 8% LiCl solution in *N*,*N*-dimethylacetamide (DMAC) HPLC grade at 70–80 °C and further diluted with DMAC to a concentration of about 0.4%. The SEC analysis was carried out by PL-GPC 110 system (Polymer Laboratories, UK) equipped with four PLgel 10 μm MIXED B 300 × 7.5 mm columns and pre-column type PLgel 10 μm. The columns, injector and Infrared detector were maintained at 80 °C during the analysis. The eluent (0.5% LiCl solution in DMAC) was pumped at a flow rate of 1.0 mL min^{-1}. The analytical columns were calibrated with pullulan standards (Polymer Laboratories) in the range 0.8–100 kDa.

Hydrogel synthesis

Xylan-based hydrogels were prepared through a three-step procedure.[34,55,56] The first step involves the preparation of 2-[(1-imidazolyl)formyloxy]ethyl methacrylate (HEMA-Im) which was synthesized as already reported by Ranucci *et al.*[57] by reacting 20.3 g (156 mmol) of 2-hydroxyethyl methacrylate (HEMA) with 50.67 g (312 mmol) of *N*,*N*′-carbonyldiimidazole (CDI) in 80 mL of anhydrous $CHCl_3$ at room temperature for 1 h. The organic phase was then neutralized and washed with several portions of water and dried over Na_2SO_4 before removing the solvent. HEMA-Im was, in the second step, covalently coupled with the xylans. Briefly, 1.5 g of each xylan and 2.0 g of HEMA-Im were dissolved in 60 mL of DMSO under stirring. 250 μL of triethylamine was added to initiate the reaction and the mixture was left at 50 °C under stirring for a time period between 6 and 120 h depending on the targeted degree of substitution (DS). The product was precipitated in ethyl acetate, extensively washed and centrifuged and finally dried. The DS was determined by ^{1}H-NMR spectroscopy as described below (see Table 1).

Finally, xylan-based hydrogels were prepared with different compositions using HEMA as a co-monomer. Hydrogels with two weight ratio of xylans to HEMA (60 : 40 and 40 : 60) were prepared. In a typical experiment, prescribed amount of xylans was dissolved in a small volume of deionized water and the corresponding amount of HEMA was added, and the resulting mixture then was thoroughly stirred. Catalytic amounts of water solutions of ammonium peroxodisulfate and sodium pyrosulfite were then added to initiate the cross-linking and solution was

transferred quickly to a cylindrical mold before gelation. The mold was sealed with Parafilm™ and the mixture was left at room temperature for at least 6 h before analysis. The incorporation of doxorubicin was realized simultaneously with the cross-linking by adding the desired amount of doxorubicin to the mixture before initiating the cross-linking. No crosslinking of the doxorubicin was detected based on the chemistry of the crosslinking reaction and gravimetric experiments.

^{1}H-NMR measurements

Modified and unmodified xylans were dissolved in D_2O or DMSO-d_6. Spectra were recorded on an AC 500 MHz Bruker spectrometer at 303 K in a 5 mm o.d. tube (internal acetone ^{1}H (CH_3) at 2.1 ppm relative to Me_4Si). The DS of modified xylans was determined from ^{1}H-NMR spectra. For that, the area of the acetyl peak (δ 2.0 ppm—3H), 4-*O*-methyl glucuronic acid peak (4-*O*-MeGlcA) (δ 5.3 ppm—1H) and vinyl C–H peaks (δ 5.71 and δ 6.05 ppm—1H each) was integrated; the obtained value for the vinyl peaks was related to the acetyl peak for acetylated xylan and to the 4-*O*-MeGlcA peak for non-acetylated xylan. The DS of the non-acetylated xylan was determined in D_2O due to the quality of the 4-*O*-MeGlcA peak.

Hydrogel swelling ratio measurements

Gels were previously freeze-dried, and then they were immersed in an excess of deionized water at 37 °C. At various time, the samples were withdrawn from the water medium and weighted. The swelling, Q_S, was then calculated from: $Q_S = (W_s - W_d)/W_d$, where W_d is the weight of the dry gel prior to swelling and W_s is the weight at the swollen state.

Scanning Electron Microscopy (SEM)

The morphology of the hydrogels was controlled by field emission scanning electron microscopy (FE-SEM) using a JEOL 6400F microscope operated with an accelerating voltage of 5 kV and a working distance of 15 mm, and a 30 μm objective aperture. A small hydrogel sized sample was affixed onto a conductive carbon tape and mounted on the support and then sputtered with an approximately 25 nm layer of gold/palladium (60/40).

Bounded water content

Non-freezable bound water content (W) in the swollen hydrogel was determined using differential scanning calorimetry (TA Instruments DSC Q100). Portions of hydrogels (5–10 mg) were cut from the pre-equilibrated swollen gels, placed in the pre-weighted aluminium pan, sealed, cooled to −20 °C and maintained at this temperature for 5 min. The temperature was raised to −10 °C at a heating rate of 1 °C per min and the sample was maintained isothermally until the heat flow returned to the baseline value. Subsequent heating steps to higher temperatures (−5, 0, 5 °C) were then successively performed. The free and freezable water content (W_f) was calculated from the sum of the endothermic enthalpies corresponding to the melting of water by using the following equation:

$$W_f = \Delta H \times (1 + W_t)/\Delta H_w$$

where W_f is the free and freezable water content, W_t is the total water content estimated by gravimetry after swelling of the gels, ΔH is the sum of enthalpies from the DSC thermogram. The value of ΔH_w is the melting enthalpy of pure water at the corresponding fusion temperature and can be estimated using the following equation:

$$\Delta H_w = \Delta H_w^0 - \Delta c_p \Delta T$$

where ΔH_w^0 is the pure water enthalpy at 0 °C, Δc_p is the specific heat capacity between the liquid water and solid ice, ΔT is the freezing point depression. Non-freezable bound water content (W_b) was estimated by subtracting the free and freezable water content from the total water content: $W_b = W_t - W_f$

Rheological properties

Viscoelastic measurements for determination of G' (shear storage modulus) and G'' (shear loss modulus) were performed on a StressTech model rheometer (Reologica Instruments). Cylindrical discs (diameter = 8 mm and height = 4 mm) were cut from the hydrogels as prepared and the experiments were carried out by using a parallel plate geometry with a diameter of 8 mm. A dynamic frequency sweep test was performed at 25 °C with each sample at 5% of strain within a frequency range from 10 to 0.1 Hz and 10 to 0.01 Hz for hydrogels made from acetylated xylans and non-acetylated xylans, respectively.

Doxorubicin release from xylan-based hydrogels

Drug release analysis from the hydrogels was performed after immersing the samples in a water bath at 37 °C under 50 rpm stirring and where the pH was previously adjusted to 2.5 and 7. Samples were withdrawn at different time intervals over a time period of 50 h of the gel to remove spontaneously released doxorubicin. The released doxorubicin was measured using a Lambda 3B UV/VIS spectrophotometer (Perkin Elmer, Norwalk, CT) at 486 nm.

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References

1 J. Jagur-Grodzinski, *Polym. Adv. Technol.*, 2010, **21**, 27–47.
2 W. Cai and R. B. Gupta, in*Kirk-Othmer Encyclopedia of Chemical Technology*, ed. S. Arza, John Wiley & Sons, Inc, Hoboken, NJ, 5th edn, 2005, vol. 13, pp. 729–754.
3 T. R. Hoare and D. S. Kohane, *Polymer*, 2008, **49**, 1993–2007.
4 K. Pal, A. K. Banthia and D. K. Majumdar, *Des. Monomers Polym.*, 2009, **12**, 197–220.

5 B. V. Slaughter, S. S. Khurshid, O. Z. Fisher, A. Khademhosseini and N. A. Peppas, *Adv. Mater.*, 2009, **21**, 3307–3329.
6 J. K. Oh, D. I. Lee and J. M. Park, *Prog. Polym. Sci.*, 2009, **34**, 1261–1282.
7 M. S. Lindblad, J. Sjoeberg, A.-C. Albertsson and J. Hartman, in *Materials, Chemicals, and Energy from Forest Biomass*, ed. D. S. Argyropoulos, American Chemical Society, Washington, DC, 2007, vol. 954, pp. 153–167.
8 M. Giannuzzo, M. Feeney, P. Paolicelli and M. A. Casadei, *J. Drug Delivery Sci. Technol.*, 2006, **16**, 49–54.
9 W. N. E. van Dijk-Wolthuis, J. A. M. Hoogeboom, M. J. van Steenbergen, S. K. Y. Tsang and W. E. Hennink, *Macromolecules*, 1997, **30**, 4639–4645.
10 S. R. Van Tomme and W. E. Hennink, *Expert Rev. Med. Devices*, 2007, **4**, 147–164.
11 A. Barbetta, E. Barigelli and M. Dentini, *Biomacromolecules*, 2009, **10**, 2328–2337.
12 H. Park, S.-W. Kang, B.-S. Kim, D. J. Mooney and K. Y. Lee, *Macromol. Biosci.*, 2009, **9**, 895–901.
13 H. Park and K. Y. Lee, in *Natural-based polymers for biomedical applications*, ed. R. L. Reis, CRC Press, Minho, 2008, ch. 4, pp. 515–532.
14 R. A. A. Muzzarelli and C. Muzzarelli, in *Handbook of Hydrocolloids*, ed. G. O. Phillips and P. A. Williams, Woodhead Publishing Ltd., Cambridge, UK, 2nd edn, 2009, pp. 849–888.
15 N. Bhattarai, J. Gunn and M. Zhang, *Adv. Drug Delivery Rev.*, 2010, **62**, 83–99.
16 C. Jarry and M. S. Shive, in *Smart Materials*, ed. M. Schwartz, CRC Press, Boca Raton, 2009, ch. 10, pp 10/13–10/20.
17 Y. A. Aggour, *Starch/Staerke*, 1993, **45**, 55–59.
18 W. M. Kulicke, Y. A. Aggour, H. Nottelmann and M. Z. Elsabee, *Starch/Staerke*, 1989, **41**, 140–146.
19 S. Tanodekaew, S. Channasanon and P. Uppanan, *J. Appl. Polym. Sci.*, 2006, **100**, 1914–1918.
20 U. Edlund and A. C. Albertsson, *J. Bioact. Compat. Polym.*, 2008, **23**, 171–186.
21 M. S. Lindblad, E. Ranucci and A.-C. Albertsson, *Macromol. Rapid Commun.*, 2001, **22**, 962–967.
22 J. Voepel, J. Sjoeberg, M. Reif, A.-C. Albertsson, U.-K. Hultin and U. Gasslander, *J. Appl. Polym. Sci.*, 2009, **112**, 2401–2412.
23 A. M. Stephen, in *The Polysaccharides*, ed. G. O. Aspinall, Academic Press, Orlando, 1983, pp. 98–193.
24 K. C. B. Wilkie, *Adv. Carbohydr. Chem. Biochem.*, 1979, **36**, 215–264.
25 A. Ebringerova and Z. Hromadkova, *Biotechnol. Genet. Eng. Rev.*, 1999, **16**, 325–346.
26 M. Hashi and T. Takeshita, *Agric. Biol. Chem.*, 1979, **43**, 951–959.
27 M. Hashi and T. Takeshita, *Agric. Biol. Chem.*, 1979, **43**, 961–967.
28 I. Gabrielii and P. Gatenholm, *J. Appl. Polym. Sci.*, 1998, **69**, 1661–1667.
29 I. Gabrielii, P. Gatenholm, W. G. Glasser, R. K. Jain and L. Kenne, *Carbohydr. Polym.*, 2000, **43**, 367–374.
30 S. K. Sengupta, in *Cancer Chemotherapeutic Agents*, ed. W. O. Foye, American Chemical Society, Washington, DC, 1995, pp. 205–218.
31 D. Evtuguin, J. Tomás, A. S. Silva and C. Neto, *Carbohydr. Res.*, 2003, **338**, 597–604.
32 A. d. S. Magaton, D. Piló-Veloso and J. L. Colodette, *Quim. Nova*, 2008, **31**, 1085–1088.
33 A. Teleman, J. Lundqvist, F. Tjerneld, H. Stålbrand and O. Dahlman, *Carbohydr. Res.*, 2000, **329**, 807–815.
34 A. A. Roos, U. Edlund, J. Sjöberg, A.-C. Albertsson and H. Stålbrand, *Biomacromolecules*, 2008, **9**, 2104–2110.
35 W. N. E. van Dijk-Wolthuis, M. J. van Steenbergen, W. J. M. Underberg and W. E. Hennink, *J. Pharm. Sci.*, 1997, **86**, 413–417.
36 J. Voepel, J. Sjöberg, M. Reif, A.-C. Albertsson, U.-K. Hultin and U. Gasslander, *J. Appl. Polym. Sci.*, 2009, **112**, 2401–2412.
37 X. Qu, A. Wirsén and A. C. Albertsson, *Polymer*, 2000, **41**, 4589–4598.
38 G. M. Kavanagh and S. B. Ross-Murphy, *Prog. Polym. Sci.*, 1998, **23**, 533–562.
39 S. B. Ross-Murphy, *Polym. Gels Networks*, 1994, **2**, 229–237.
40 S. B. Ross-Murphy, in *Polymer Networks: Principles of Formation, Structure and Properties*, ed. R. F. T. Stepto, Chapman & Hall, Glasgow, 1998, pp. 290-318.
41 K. S. Anseth, C. N. Bowman and L. Brannon-Peppas, *Biomaterials*, 1996, **17**, 1647–1657.
42 M. Söderqvist Lindblad, A.-C. Albertsson, E. Ranucci, M. Laus and E. Giani, *Biomacromolecules*, 2005, **6**, 684–690.
43 H. Chanzy, M. Dube and R. H. Marchessault, *Polymer*, 1979, **20**, 1037–1039.
44 I. Nieduszynski and R. H. Marchessault, *Biopolymers*, 1972, **11**, 1335–1344.
45 H. Saito, J. Yamada, Y. Yoshioka, Y. Shibata and T. Erata, *Biopolymers*, 1991, **31**, 933–940.
46 H. Saito, M. Yokoi and Y. Yoshioka, *Macromolecules*, 1989, **22**, 3892–3898.
47 H. Saito, Y. Yoshioka, M. Yokoi and J. Yamada, *Biopolymers*, 1990, **29**, 1689–1698.
48 H. Saito, M. Yokoi and J. Yamada, *Carbohydr. Res.*, 1990, **199**, 1–10.
49 K. L. Shantha and D. R. K. Harding, *Int. J. Pharm.*, 2000, **207**, 65–70.
50 P. M. de la Torre, Y. Enobakhare, G. Torrado and S. Torrado, *Biomaterials*, 2003, **24**, 1499–1506.
51 H. Saito, A. S. Hoffman and H. I. Ogawa, *J. Bioact. Compat. Polym.*, 2007, **22**, 589–601.
52 D. Evtuguin, J. Tomás, A. S. Silva and C. Neto, *Carbohydr. Res.*, 2003, **338**, 597–604.
53 E. Adler, G. Brunow and K. Lundquist, *Holzforschung*, 2009, **41**, 199–207.
54 T. Ehrman, *Method for Determination of Acid-Soluble Lignin in Biomass*, National Renewable Energy Laboratory, Golden, CO, 1996.
55 M. Soderqvist Lindblad, A.-C. Albertsson, E. Ranucci, M. Laus and E. Giani, *Biomacromolecules*, 2005, **6**, 684–690.
56 M. S. Lindblad, E. Ranucci and A.-C. Albertsson, *Macromol. Rapid Commun.*, 2001, **22**, 962–967.
57 E. Ranucci, G. Spagnoli and P. Ferruti, *Macromol. Rapid Commun.*, 1999, **20**, 1–6.