Macroporous hydrogels based on carbohydrates monomethacrylates and dimethacrylates: singular properties from carbohydrate-based crosslinkers

Giovanni Bortoloni Perin, Lucas Polo Fonseca and Maria Isabel Felisberti*

Abstract

BACKGROUND: Readily available feedstock and biocompatibility make carbohydrate-based hydrogels promising materials for biomedical applications. However, carbohydrate-based crosslinkers are rather underexplored when compared with crosslinkers derived from fossil resources. In this study, novel fully bio-based hydrogels derived from enzymatically produced D-fructose and D-glucose methacrylate monomers were synthesized with different amounts of D-fructose dimethacrylate crosslinker.

RESULTS: The use of a carbohydrate-based crosslinker endows hydrogels with high swelling coefficients, up to 2400%, and superior mechanical resistance (compressive modulus up to 9.5 kPa with a maximum stress up to 50 kPa) compared with conventional crosslinkers based on fossil resources. Hydrogels shape and crosslinking density influences hydrogel morphology, swelling behavior and mechanical resistance. Moreover, hydrogels presented cell viability, biodegradability and hydrolysis-resistance over a wide range of pH.

CONCLUSION: The use of a highly hydrophilic crosslinker based on carbohydrate for hydrogels synthesis enables the use of high crosslinker concentration, which improves mechanical properties, however with minor loss of the water swelling capacity, compared with conventional fossil-based crosslinkers. This is an important advantage over conventional crosslinkers based on fossil resources. Moreover, slab hydrogels hold higher stress under compression–decompression cycles, and present higher resistance to hydrolysis in basic medium due to the thicker pore walls than cylindrical ones.

Supporting information may be found in the online version of this article.

Keywords: hydrogels; carbohydrate monomers; carbohydrate-based crosslinker; mechanical properties; biocompatibility; biodegradation

INTRODUCTION

Carbohydrates are readily available renewable raw materials with a wide range of compositions and stereochemistry. Therefore, chemical modifications of carbohydrates for monomer production comprise of a relevant chemical, economic and environmentally friendly strategy to supply new polymeric materials to modern society.1,2

Since the synthesis of carbohydrate-based polymers and hydrogels by the chemo-enzymatic route reported by Dordick and coworkers,3–5 many monomers, crosslinkers and polymeric materials based on carbohydrates (named glycopolymers) have been prepared.6–9 In this context, our research group has been investing great efforts in developing new polymeric materials based on carbohydrates, such as polymers, copolymers and hydrogels based on fructose, glucose and sucrose methacrylates.10–14

The carbohydrate moieties in glycopolymers are capable of playing some specific biological interactions, such as interactions between glucose moieties with the lectin Concanavalin A15–17 and interactions between fructose moieties with the glucose receptor GLUT5 (receptor overexpressed in the cell membrane of mammalian cancer cells).18–20 Moreover, hydrophilic polymers such as polysaccharides and glycopolymers present anti-fouling properties that diminish protein and bacterial adhesion, avoiding platelet adhesion, inflammation, device failure, blood clots, and so on.21–24 All features mentioned earlier make glycopolymers very promising materials for biomedical applications.6,25–27

The polymerization of carbohydrate-based monomers in the presence of a crosslinker results in tridimensional polymer networks, which, in general, present high water swelling capability and dimensional stability. The water in the swollen networks or hydrogels acts as plasticizer, increasing the polymer chains flexibility, endowing elasticity and soft rubbery consistency.28,29

Applications reported in the literature for hydrogels based on...
carbohydrate are mainly related to drug delivery systems. However, they have also been used as stationary phase in high-performance liquid chromatography and as cell culture media.

Crosslinking agents play a key role on the mechanical and physical chemical properties of the hydrogels. For reversible physical crosslinked hydrogels, intermolecular interactions are responsible for the network formation, while for irreversible chemical crosslinked hydrogels, the network results from covalent bonds. Crosslinker may also influence hydrogel pH-sensitivity, for instance. However, the replacement of conventional crosslinker based on fossil resources to those based on carbohydrates avoids or minimizes cytotoxicity.

Nevertheless, synthesis of hydrogels fully based on carbohydrate monomers and crosslinkers are still barely reported. Some advantages of using carbohydrate-based crosslinkers are related to their high hydrophilicity, biocompatibility, biodegradability, and similar reactivity compared with carbohydrate-based monomers, avoiding network heterogeneities due to the formation of domains with different crosslinking density.

In general, carbohydrate-based crosslinking agents have been synthesized by multi-step chemical routes or by one-step enzymatic routes. The enzymatic routes for functionalization of carbohydrates lead mainly to monofunctionalized and difunctionalized carbohydrates, allowing the simultaneous synthesis of monomers and crosslinker.

In a previous study, we reported an enzymatic route for the synthesis of fructose methacrylate (FMA) and glucose methacrylate (GMA) monomers, as well as of the crosslinking agents fructose dimethacrylate (FdMA) and glucose dimethacrylate (GdMA), based on the transesterification between 2,2,2-trifluoroethyl methacrylate (TFMA) and carbohydrate catalyzed by lipase acrylic resin (expressed in Aspergillus niger) from Candida antarctica lipase B (EC 3.1.1.3). Moreover, some hydrogels based on FMA and crosslinked with ethylene glycol dimethacrylate (EGdM) have also been prepared and characterized in relation to swelling behavior and thermal, mechanical and morphological properties.

In this study, we report the synthesis of hydrogels that are fully based on the carbohydrates methacrylates via the polymerization of FMA and GMA monomers in the presence of the FdMA crosslinker. For each monomer (FMA and GMA) three different FdMA concentrations were used (0.5 mol%, 1.0 mol% and 2.0 mol%, in relation to monomers) to prepare cylindrical and slab hydrogels, by radical polymerization. These hydrogels were properly characterized in relation to their structure, morphology, swelling behavior in aqueous media, thermal and mechanical properties, hydrolytic stability, biodegradation and cytotoxicity. Moreover, the fractions of freezable and non-freezable water in the hydrogels were estimated. Our study enables us to understand the influence of the crosslinking density, shape and morphology of the hydrogels on their properties. Moreover, the advantages of using a carbohydrate-based crosslinker are highlighted.

MATERIALS AND METHODS

Materials

Lipase acrylic resin (expressed in Aspergillus niger) from Candida antarctica lipase B (EC 3.1.1.3), TFMA, dimethylsulfoxide (DMSO) and tert-butanol were purchase from Sigma-Aldrich (Darmstadt, Germany). D-Fructose, D-glucose, dimethylformamide (DMF), ammonium persulfate (APS), chloroform and methanol were purchased from Labsynth (Diadema, Brazil). All these reagents of analytical grade were used without any further treatment or purification. Protease Protin® NY100 kindly provided by Amano (Nagoya, Japan) was treated with an aqueous solution of potassium hydroxide (KOH) at pH 10, followed by freeze-drying, as described elsewhere. Dulbecco’s modified Eagle's medium (DMEM), penicillin/streptomycin solution and fetal bovine serum was purchased from Sigma-Aldrich.

In this study, we report the synthesis of hydrogels that are fully based on the carbohydrates methacrylates via the polymerization of FMA and GMA monomers in the presence of the FdMA crosslinker. For each monomer (FMA and GMA) three different FdMA concentrations were used (0.5 mol%, 1.0 mol% and 2.0 mol%, in relation to monomers) to prepare cylindrical and slab hydrogels, by radical polymerization. These hydrogels were properly characterized in relation to their structure, morphology, swelling behavior in aqueous media, thermal and mechanical properties, hydrolytic stability, biodegradation and cytotoxicity. Moreover, the fractions of freezable and non-freezable water in the hydrogels were estimated. Our study enables us to understand the influence of the crosslinking density, shape and morphology of the hydrogels on their properties. Moreover, the advantages of using a carbohydrate-based crosslinker are highlighted.

Synthesis and purification of hydrogel

In a typical hydrogel synthesis, a 34 wt% solution of FMA, or GMA monomer in deionized water/DMF (1.0:0.9 m/m) containing 1.0 mol% of APS and FdMA crosslinker at different amounts (0.5, 1.0 or 2.0 mol% in relation to the monomer), was purged with argon for at least 10 min, and then transferred to a suitable container where the reaction takes place at 60 °C for 6 h. For the hydrogels synthesized in the cylindrical shape, the container used was a 1.5 mL Eppendorf tube and for the synthesis of hydrogels in the slab shape the container used was a Teflon mold trapped between two metal plates and fastened using screws. The hydrogels were purified prior to the characterizations by immersion in deionized water, for at least three days changing the water every day, to remove the solvent DMF and residual monomers. Hydrogels were freeze-dried producing xerogels. The nomenclature to identify hydrogels and xerogels is ‘PFMA X mol% FdMA’ for poly(fructose methacrylate) crosslinked with X mol% of the crosslinker FdMA (where X = 0.5, 1.0 or 2.0). In a similar way, ‘PGMA X mol% FdMA’ is used for poly(glucose methacrylate) hydrogels and xerogels. Both hydrogels and xerogels were characterized as outlined later.

Characterization

For the proton nuclear magnetic resonance (1H-NMR), xerogels were ground and putted into the NMR tube with deuterated water (D2O). After 2 h swelling, the analysis was performed in a Bruker equipment model Avance 500 MHz (Billerica, Massachusetts) operating at 25°C and 11.7 Tesla, with a pulse delay of 1 s, an acquisition time of 1.6 s, 128 scans with 32 k points and a free induction decay (FID) resolution of 0.3 Hz. Chemical shifts (δ) in parts per million (ppm) were assigned regarding the D2O residual solvent proton signal at δ = 4.79 ppm.

The thermal properties of the xerogels were determined by differential scanning calorimetry (DSC) on a DSC Q2000 TA Instruments (New Castle, Delaware). For these analysis, 5–10 mg of the xerogel were hermetically sealed in aluminum pans and analyzed according to the following program: (i) equilibrium at 25 °C; (ii) heating to 80 °C at 20 °C min−1; (iii) cooling to −100 °C at 20 °C min−1; (iv) isotherm for 5 min; (v) heating to 150 °C at 20 °C min−1.

The thermal stability of the xerogel was evaluated by thermogravimetric analysis (TGA) using a TGA 2950 TA instruments thermo-balance under argon atmosphere (flow of 100 mL min−1). Samples of 5 to 10 mg of xerogels were heated in a temperature range from 30 to 600 °C, at a heating rate of 10 °C min−1.

The inner hydrogel morphology was investigated by scanning electron microscopy (SEM) analysis. For these analyses, xerogels...
were swollen in an aqueous solution of 1.0% (v/v) DMSO and then freeze-dried. The freeze-dried samples were cut into a suitable size, fixed on a sample holder with graphite tape and metallized with iridium in a Bal-Tec MD 020 metallizer (Wetzlar, Germany). A QUANTA 250 FEG scanning electron microscope (Hillsboro, Oregon) operating at 6.00 kV under a vacuum was used to acquire the images.

To determine the swelling coefficient at equilibrium, 50–80 mg of xerogels were placed in an aqueous medium at 25 °C and, after 24 h (time enough for samples to achieve the swelling equilibrium, as observed from swelling kinetics data shown in Supporting Information Fig. S1), they were taken from the medium, superficially dried and weighed in an analytical balance. The aqueous media used in these studies were deionized water and buffer solutions at pH 2, pH 7.4 and pH 10. The swelling coefficient (Q) was calculated as:

\[
Q = \frac{m_{\text{swollen}}}{m_{\text{xerogel}}} \times 100
\]  

where \(m_{\text{swollen}}\) is the mass of the swollen hydrogel and \(m_{\text{xerogel}}\) is the mass of the xerogel.

The water content (W) at equilibrium for hydrogels PFMA and PGMA swollen in deionized water after 24 h were calculated using Eqn (2):

\[
W = \left( \frac{m_{\text{swollen}} - m_{\text{xerogel}}}{m_{\text{xerogel}}} \right) \times 100 = \left( \frac{m_{\text{water}}}{m_{\text{xerogel}}} \right) \times 100
\]  

The fraction of freezable water (\(f_{\text{fw}}\)) was estimated from DSC data. For the analysis, 5–10 mg of previously swollen hydrogels (\(W\)) were calculated using Eqn (2):

\[
f_{\text{fw}} = \frac{\Delta H_m}{\Delta H_w} \times 100
\]  

where \(\Delta H_m\) is the melting enthalpy of the water in the hydrogels and \(\Delta H_w\) is the standard melting enthalpy of pure water (\(\Delta H_w = 334 \text{ J g}^{-1}\)).

The fraction of non-freezable water (\(f_{\text{nfw}}\)) was estimated by the difference between the water content into the swollen hydrogel (\(W\)) and the fraction of freezable water (\(f_{\text{fw}}\)):

\[
f_{\text{nfw}} = W - f_{\text{fw}}
\]

The mechanical properties of swollen cylindrical (2.5–5.0 mm height and 7–10 mm diameter) and slab (1.4–2.0 mm height and 5.0 mm diameter) shaped hydrogels at 25 °C were evaluated by compression tests using the thermomechanical analyzer TMA 2940 TA Instruments equipment according to the following program: (i) equilibrium at 25 °C for 5 min; (ii) cooling to −30 °C at 2 °C min⁻¹; (iii) isotherm for 5 min; (iv) heating to 30 °C at 2 °C min⁻¹. The \(f_{\text{fw}}\) value was calculated as follows:

\[
f_{\text{fw}} = \frac{\Delta H_m}{\Delta H_w} \times 100
\]  

where \(\Delta H_m\) is the melting enthalpy of the water in the hydrogels and \(\Delta H_w\) is the standard melting enthalpy of pure water (\(\Delta H_w = 334 \text{ J g}^{-1}\)).

The swelling coefficient of PFMA and PGMA hydrogels in cylindrical shape was also determined at temperatures of 35 and 45 °C in a buffer solution pH 7.4 after a period of 24 h immersion at each temperature.

The hydrolytic stability of PFMA and PGMA hydrogels in slab shape was evaluated by determining the swelling coefficient of these hydrogels over a period of 12 weeks in buffers solution at pH 2, pH 7.4 and pH 10 at 25 °C.

PFMA 1.0 mol% FdMA and PGMA 1.0 mol% FdMA hydrogel cytotoxicity assays conducted against mouse fibroblasts NIH3T3 cells and evaluated by the MTS method. DMEM supplemented with 10% of fetal bovine serum, 100 μg L⁻¹ streptomycin and 100 IU mL⁻¹ penicillin were used to cultivate the cells under a 5.0% carbon dioxide (CO₂)/95% air atmosphere in an incubator. Cells were seeded in a 96-well plate at a concentration of 10⁴ cells per well with 200 μL of culture medium. After 24 h, 1.0 mg of grounded xerogel swollen with culture medium was added in the wells and left to incubate for another 24 h. Positive control was conducted with 200 μL of culture medium for 48 h. Cytotoxicity assays were also conducted with the culture medium exposed to the xerogels, for one month, at 8 °C to evaluate the cytotoxicity of degradation products of the xerogels. After 24 h of incubation the culture medium was replaced for 200 μL of culture medium exposed to the xerogels and cells were left to incubate for another 24 h. Positive control was conducted by changing the culture medium for a fresh one after 24 h of incubation. Then 10 μL of MTS reagent was added to the wells and left to incubate for 2 h. The absorbance at 492 nm was measured in a multiplate reader Perkin Elmer (Waltham, Massachusetts). All assays were conducted in quintuplicate. The number of cells were expressed as percent of viability where \(A_i\) is the absorbance of the treated wells, \(A_o\) is the absorbance of positive control wells and \(A_o\) the absorbance of culture medium. Error bars were calculated as the standard deviation of the percent viability.

RESULTS AND DISCUSSION

Structural characterization

The chemical structures of PGMA and PFMA networks were confirmed by 1H-NMR. The spectra of the networks prepared with 0.5 mol% of FdMA (Fig. 1) present overlapping peaks at around 3.0–4.5 ppm assigned to the hydrogens H₁, H₂, H₃, H₄ and H₅ of the carbohydrates pendant groups and signals around 1.0 and 2.0 ppm assigned to H₆ and H₇ of methyl hydrogens of the methacrylic and the methylene hydrogens of the polymeric backbone, respectively. Signals related to FdMA crosslinker also appear overlapped in the hydrogels spectra at around 3.0–4.5 ppm. The absence of vinylic hydrogens in the range 5.5–6.5 ppm, confirms the polymerization of the monomers and the hydrogel purity, as described elsewhere.

Xerogel characteristics

PFMA and PGMA xerogels, both cylindrical and slab-shaped, were analyzed regarding their thermal properties by DSC and TGA.
Figure 1. $^1$H-NMR spectra in D$_2$O: (a) PFMA and (b) PGMA networks.

Figures S2 and S3 show the DSC second heating scans and Fig. S4 shows the xerogel thermal degradation profile, as determined by TGA. The thermal properties are summarized in Table 1.

Xerogels are amorphous and present a broad glass transition ($\Delta T_g$ varying from 40 to 90 °C) and glass transition temperature ($T_g$), varying in the range from 94 to 120 °C. Oliveira and Felisberti$^{10}$ reported the glass transition temperature of the amorphous poly(sucrose methacrylate) at 116 °C, while Zakhireh et al.$^{30}$ reported $T_g$ values of polymer networks based on allyl $\alpha$-D-galactopyranoside and methacrylic acid crosslinked with 1,6-hexanediol diacylate or 1,6-hexanediol propoxylate diacylate around 170–190 °C. The high $T_g$ of these polymers has been attributed to the high density of intermolecular interactions among the pendant groups. Besides this, the high $T_g$ values observed for the PFMA and PGMA xerogels can also be addressed to the crosslinking, which decreases the polymer chain flexibility.

The thermal stability of hydrogels was investigated by TGA and both PFMA and PGMA xerogels present three degradation stages under an inert atmosphere. The first and second stages are related to carbohydrate degradation,$^{47,48}$ and the third stage is related to the backbone depolymerization and random cleavage.$^{10}$ The PFMA and PGMA xerogels present initial thermal degradation at around 155 °C and 175 °C, respectively, following the same tendencies observed for the fructose and glucose pyrolyses.$^{49}$

SEM micrographs of PFMA and PGMA xerogels prepared by freeze-drying of the corresponding hydrogels, Figs 2 and 3 respectively, show that independently of the shape (cylinder or slab), all hydrogels present a porous inner structure with millimetric dimensions, being classified as macroporous materials.$^{50}$ A slight tendency towards a decrease in the pore size with an increase in crosslinking density is observed. Moreover, slab-shaped hydrogels present a thicker pore wall than cylindrical ones, which may be an effect of the mold used in the polymerization.

Hydrogel characterization

Table 2 summarizes the mechanical properties and swelling behavior of PFMA and PGMA hydrogel.

The presence of carbohydrate moieties and the use of the hydrophilic FdMA crosslinker endow the hydrogel high swelling capability in aqueous media. In general, these hydrogels prepared in an aqueous solution of monomers (34 wt%) and crosslinked with FdMA shows a swelling coefficient around three- to five-fold higher than similar hydrogels crosslinked with EGdM.$^{13}$ For example, cylindrical PFMA 0.5 mol% FdMA and PGMA 0.5 mol% FdMA present swelling coefficients of 2237 ± 183 and 1987 ± 245%, respectively, while the swelling coefficient of cylindrical PFMA 0.5 mol% EGdM is reported to be around 450%.$^{13}$ In the same way, for cylindrical PFMA 1.0 mol% FdMA and PGMA 1.0 mol% FdMA, the swelling coefficient is 1200% and for PFMA 1.0 mol% EGdM around 350%. Similar behavior has been reported by Paterson et al. for PHEMA (poly(2-hydroxyethyl methacrylate)) hydrogels crosslinked with tetraethyleneglycol dimethacrylate (less hydrophilic and fossil-based crosslinker) and carbohydrate-based crosslinker.$^{35}$
Table 1. Thermal properties of PFMA and PGMA xerogels: glass transition temperature ($T_g$), glass transition width ($\Delta T_g$), heat capacity change ($\Delta C_p$), initial thermal degradation temperature ($T_{onset}$), temperature of maximum mass loss rate ($T_{max}$) and mass loss of each degradation step

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Shape</th>
<th>$T_g$ (°C)</th>
<th>$\Delta T_g$ (°C)</th>
<th>$T_{onset}$ (°C)</th>
<th>$T_{maxI}$ (°C)</th>
<th>$T_{maxII}$ (°C)</th>
<th>Mass loss (%)</th>
<th>$T_{onset}$ (°C)</th>
<th>$T_{maxI}$ (°C)</th>
<th>$T_{maxII}$ (°C)</th>
<th>Mass loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFMA 0.5 mol% FdMA</td>
<td>Cylinder</td>
<td>94</td>
<td>66</td>
<td>155</td>
<td>221</td>
<td>237</td>
<td>32</td>
<td>343</td>
<td>424</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Slab</td>
<td>106</td>
<td>67</td>
<td>155</td>
<td>215</td>
<td>252</td>
<td>31</td>
<td>345</td>
<td>425</td>
<td>73</td>
<td></td>
</tr>
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<td>94</td>
<td>71</td>
<td>155</td>
<td>203</td>
<td>262</td>
<td>32</td>
<td>343</td>
<td>422</td>
<td>73</td>
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<tr>
<td></td>
<td>Slab</td>
<td>113</td>
<td>41</td>
<td>155</td>
<td>215</td>
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<td>425</td>
<td>73</td>
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<td>PGMA 0.5 mol% FdMA</td>
<td>Cylinder</td>
<td>95</td>
<td>46</td>
<td>173</td>
<td>241</td>
<td>298</td>
<td>33</td>
<td>351</td>
<td>413</td>
<td>71</td>
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<tr>
<td></td>
<td>Slab</td>
<td>103</td>
<td>64</td>
<td>176</td>
<td>242</td>
<td>299</td>
<td>32</td>
<td>353</td>
<td>426</td>
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<td>Cylinder</td>
<td>103</td>
<td>75</td>
<td>176</td>
<td>242</td>
<td>299</td>
<td>32</td>
<td>353</td>
<td>426</td>
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<td></td>
<td>Slab</td>
<td>110</td>
<td>56</td>
<td>176</td>
<td>242</td>
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<td>33</td>
<td>351</td>
<td>424</td>
<td>75</td>
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</tr>
</tbody>
</table>

$\Delta T_g = T_{endset} - T_{onset}$.

Note: PFMA, poly(fructose methacrylate); PGMA, poly(glucose methacrylate); FdMA, fructose dimethacrylate.

PFMA and PGMA hydrogels crosslinked with FdMA also present superior swelling capability (above 700% for hydrogels containing 2.0 mol% of FdMA) than other hydrogels based on carbohydrate monomers reported in the literature. For instance, hydrogels prepared in aqueous solution (concentration not specified) of glucose acrylamide and crosslinked with 0.1% w/v (0.01 mol%) of glucose bisacrylamide present a swelling coefficient of 407%,\textsuperscript{15} while hydrogels prepared in aqueous solution at 15 wt% of methylgalactose acrylate and crosslinked with 0.1 mol% of methylgalactose diacylate showed a swelling ratio at equilibrium of 1103%.\textsuperscript{28} However, hydrogels based on disaccharide monomers, such as sucrose (meth)acrylates, and crosslinked with a sucrose-based crosslinker tends to present higher swelling coefficient, reaching values higher than 2500% as reported by de Menezes \textit{et al.}\textsuperscript{12} and by Patil \textit{et al.},\textsuperscript{44} for crosslinker concentrations of 1.0 mol% and 2.0 mol%, respectively.

Swelling coefficients for PFMA and PGMA hydrogels decrease with increasing crosslinking density, as expected. Moreover, swelling coefficient decreases significantly in the buffered medium, compared with deionized water, probably due to ionic strength, as reported for poly(2-hydroxyethyl methacrylate) hydrogels.\textsuperscript{51-53} The knowledge of the swelling behavior of hydrogels in electrolyte solution is of great importance because hydrogels are mainly used in biomedical applications. The contact...
of hydrogels with biological fluids can be a trigger for hydrogel deswelling and controlled drug release.

Figure 4 shows the stress–strain curves for cylindrical and slab-shaped hydrogels (Fig. 4(a) and (c), respectively) obtained in the cyclic compression–decompression test, and maximum stress (σ\text{max}) as a function of maximum strain (ε\text{max}) for PFMA and PGMA hydrogels (Fig. 4(b) and (d), respectively), for each cycle with crescent applied stress. Figure S5 shows, as an example, a full stress–strain curve for the cyclic compression–decompression test of PGMA 2.0 mol% FdMA.

In general, higher crosslinking density leads to more resistant and stiffer hydrogels, as can be observed by the increase in the compressive modulus, in the stress-at-break and by the decrease in the strain-at-break (Table 2). Slab hydrogels tends to have a relative smaller maximum strain (for a similar maximum stress) than cylindrical shaped hydrogels and to hold stress up to 50 kPa without rupture, while the cylindrical hydrogels holds stress up to 25 kPa (Fig. 4(b) and (d)). These differences results from the hydrogel morphology, slab shape hydrogels present thicker pore wall than the cylindrical ones. Moreover, because polymerization occurs from the outside in, hydrogels presents a dense skin, as outlined in Fig. 5. The surface/volume ratio is higher for slab-shaped hydrogels making them mechanically more resistant compared with cylindrical ones.

The use of FdMA as crosslinker leads to hydrogels with higher hydrophilicity, higher swelling capability and, at same time, higher mechanical resistance compared with hydrogels crosslinked with EGdM. For example, the cylindrical hydrogel PFMA 2.0 mol% FdMA

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Shape</th>
<th>Q pH 2 (%)</th>
<th>Q pH 7.4 (%)</th>
<th>Q pH 10 (%)</th>
<th>E (kPa)</th>
<th>ε at break (%)</th>
<th>σ at break (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFMA 0.5 mol% FdMA Cylinder</td>
<td>2237 ± 183</td>
<td>1383 ± 244</td>
<td>1223 ± 28</td>
<td>1769 ± 327</td>
<td>1.6</td>
<td>128</td>
<td>3.1</td>
</tr>
<tr>
<td>Slab</td>
<td>1736 ± 82</td>
<td>1323 ± 70</td>
<td>1316 ± 44</td>
<td>1251 ± 48</td>
<td>2.3</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>PFMA 1.0 mol% FdMA Cylinder</td>
<td>1220 ± 122</td>
<td>884 ± 97</td>
<td>872 ± 50</td>
<td>1316 ± 89</td>
<td>3.1</td>
<td>106</td>
<td>6.9</td>
</tr>
<tr>
<td>Slab</td>
<td>1282 ± 16</td>
<td>953 ± 15</td>
<td>1008 ± 34</td>
<td>949 ± 3</td>
<td>4.1</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>PFMA 2.0 mol% FdMA Cylinder</td>
<td>752 ± 82</td>
<td>654 ± 27</td>
<td>643 ± 10</td>
<td>909 ± 81</td>
<td>8.8</td>
<td>97</td>
<td>18.8</td>
</tr>
<tr>
<td>Slab</td>
<td>954 ± 38</td>
<td>809 ± 4</td>
<td>837 ± 9</td>
<td>884 ± 81</td>
<td>9.5</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>PGMA 0.5 mol% FdMA Cylinder</td>
<td>1987 ± 245</td>
<td>939 ± 41</td>
<td>1040 ± 33</td>
<td>1826 ± 158</td>
<td>4.0</td>
<td>37</td>
<td>2.6</td>
</tr>
<tr>
<td>Slab</td>
<td>2340 ± 108</td>
<td>1349 ± 43</td>
<td>1360 ± 51</td>
<td>1445 ± 37</td>
<td>3.0</td>
<td>158</td>
<td>30.6</td>
</tr>
<tr>
<td>PGMA 1.0 mol% FdMA Cylinder</td>
<td>1207 ± 175</td>
<td>833 ± 12</td>
<td>900 ± 4</td>
<td>1656 ± 52</td>
<td>5.6</td>
<td>95</td>
<td>11.5</td>
</tr>
<tr>
<td>Slab</td>
<td>1429 ± 22</td>
<td>1096 ± 26</td>
<td>1067 ± 1</td>
<td>1064 ± 46</td>
<td>4.7</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>PGMA 2.0 mol% FdMA Cylinder</td>
<td>794 ± 40</td>
<td>635 ± 27</td>
<td>648 ± 36</td>
<td>1009 ± 4</td>
<td>8.3</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
<tr>
<td>Slab</td>
<td>708 ± 35</td>
<td>564 ± 38</td>
<td>598 ± 10</td>
<td>567 ± 25</td>
<td>6.6</td>
<td>n.b.</td>
<td>n.b.</td>
</tr>
</tbody>
</table>

Note: PFMA, poly(fructose methacrylate); PGMA, poly(glucose methacrylate); FdMA, fructose dimethacrylate; n.b., not break.

\( ^{a} \) Hydrogel swollen in deionized water. All swelling experiments were performed in triplicate.
Figure 4. First compression–decompression cycle (maximal force of 0.1 N) for PFMA and PGMA hydrogels swollen in deionized water at 25 °C in (a) cylindrical and (c) slab shapes, respectively. Maximum specific deformation at the maximum applied force of each cycle for PFMA and PGMA hydrogels in (b) cylindrical and (d) slab shapes, respectively: ■ PFMA 0.5 mol% FdMA; ● PFMA 1.0 mol% FdMA; ▲ PFMA 2.0 mol% FdMA; ⊙ PGMA 0.5 mol% FdMA; ○ PGMA 1.0 mol% FdMA; △ PFMA 2.0 mol% FdMA.

Figure 5. Schematic representation of the inner morphology of hydrogels prepared in cylindrical and slab shapes and of the compression–decompression assay performed. The enlarged detail: SEM micrographs show the dense skin of the hydrogels.
Figure 6. DSC heating scan for PFMA and PGMA hydrogels swollen with deionized water in (a) and (b) cylindrical and (c) and (d) slab shapes, respectively. For comparison the DSC heating curve of deionized water (I) is also present in each figure. II, PFMA 0.5 mol% FdMA; III, PFMA 1.0 mol% FdMA; IV, PFMA 2.0 mol% FdMA; V, PGMA 0.5 mol% FdMA; VI, PGMA 1.0 mol% FdMA; VII, PFMA 2.0 mol% FdMA.

FdMA presents a water swelling coefficient of 752 ± 82% and holds a maximum stress around 20 kPa, while cylindrical PFMA 0.25 mol% EGdM presents a similar swelling coefficient, of around 600%, and holds just a maximum stress of around 6 kPa. Moreover, when hydrogels were prepared in the slab shape, this effect is even more pronounced: the PFMA 2.0 mol% FdMA holds stress higher than 50 kPa with a higher swelling coefficient of 954 ± 38%.

Martin et al.28 and Chen et al.,5 reported a similar behavior by changing crosslinker for hydrogels based on α-methylgalactoside methacrylate and crosslinked with α-methylgalactoside dimethacrylate or N,N'-methylene bis(acrylamide), respectively. The hydrogel crosslinked with 1.0 mol% of α-methylgalactose dimethacrylate presented a swelling coefficient of 346% and an elastic modulus of 21 kPa, while the hydrogel crosslinked with 1.0 mol% N,N'-methylene bis(acrylamide) presented a swelling coefficient of 65% and an elastic modulus of 2.7 kPa.

In addition to the amount of water in the hydrogels, the estimation of the water state in the hydrogel is also of great importance, mainly for biomedical and pharmaceutical applications.54–56 The DSC heating scan of PFMA and PGMA hydrogels swollen with deionized water in cylindrical and slab shapes are present in Fig. 6.

Deionized water presents a broad melting peak with a minimum at 3.8 °C and an onset at 0.2 °C, while the melting peak of water in the hydrogels is also broad and presents lower onset temperatures (Table 3) related to the freezable bond water which slightly interacts with the polymer network.12,57,58

In general, water content (W) ranges from 87 to 96 wt% for PFMA hydrogels and from 82 to 93 wt% for PGMA hydrogels, following the tendency to decrease with the increase of crosslinking density. The major fraction of water in the hydrogels is freezable water (free water and freezable bond water) varying from 75 to 88 wt% of the water content and, the minor fraction is non-freezable water (between 5 and 13 wt% of the water content), which strongly interacts with the hydrophilic groups in the polymeric chain. Therefore, the PFMA and PGMA hydrogels are basically macroporous structures in which a hydrated polymeric phase is surrounded by a bulk-like water phase.

Ajish et al.15 reported for hydrogels based on glucose acrylamide and glucose bisacrylamide as crosslinkers a fraction of freezable water higher than 70%. For hydrogels based on PHEMA, Liu et al.51 reported a water content of around 50% with around 30% of freezable water, and, for hydrogels based on methacrylic acid, the water content reached 85% which included around 50% of freezable water.19 In general, these hydrogels present lower swelling degree than PFMA and PGMA hydrogels, which leads to a relative decrease in the fraction of freezable water.

The melting peak temperature of water confined in pores tends to decrease as the pore size in mesoporous materials decreases. The pore size and melting temperature are related by...
Hydrogels based on monomethacrylates and dimethacrylates

Table 3. Melting temperature, onset, melting peak and melting enthalpy of the water into hydrogels and hydrogel water content (W), fraction of freezable water (fFW) and non-freezable water (fNFW) in cylindrical and slab shape hydrogels swollen with deionized water.

<table>
<thead>
<tr>
<th>Hydrogel</th>
<th>Shape</th>
<th>T_onset (°C)</th>
<th>T_peak (°C)</th>
<th>ΔT (°C)</th>
<th>ΔH_w (J g⁻¹)</th>
<th>W (wt%)</th>
<th>fFW (wt%)</th>
<th>fNFW (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFMA 0.5 mol% FdMA</td>
<td>Slab</td>
<td>−2.2</td>
<td>4.0 / 1.5</td>
<td>8.1</td>
<td>293.3</td>
<td>92</td>
<td>88</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cylinder</td>
<td>−2.3</td>
<td>3.5</td>
<td>11.1</td>
<td>285.9</td>
<td>96</td>
<td>86</td>
<td>10</td>
</tr>
<tr>
<td>PFMA 1.0 mol% FdMA</td>
<td>Slab</td>
<td>−3.2</td>
<td>4.0 / 1.1</td>
<td>9.1</td>
<td>279.3</td>
<td>90</td>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Cylinder</td>
<td>−3.2</td>
<td>3.0</td>
<td>10.6</td>
<td>266.6</td>
<td>92</td>
<td>80</td>
<td>12</td>
</tr>
<tr>
<td>PFMA 2.0 mol% FdMA</td>
<td>Slab</td>
<td>−3.5</td>
<td>3.6 / 1.8</td>
<td>10.2</td>
<td>249.4</td>
<td>88</td>
<td>75</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Cylinder</td>
<td>−3.5</td>
<td>4.1 / 1.0</td>
<td>11.7</td>
<td>272.9</td>
<td>87</td>
<td>82</td>
<td>5</td>
</tr>
<tr>
<td>PGMA 0.5 mol% FdMA</td>
<td>Slab</td>
<td>−1.6</td>
<td>2.0</td>
<td>7.2</td>
<td>275.2</td>
<td>93</td>
<td>82</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Cylinder</td>
<td>−2.7</td>
<td>2.5</td>
<td>11.3</td>
<td>314.7</td>
<td>91</td>
<td>82</td>
<td>9</td>
</tr>
<tr>
<td>PGMA 1.0 mol% FdMA</td>
<td>Slab</td>
<td>−1.9</td>
<td>1.7</td>
<td>6.9</td>
<td>285.3</td>
<td>91</td>
<td>85</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Cylinder</td>
<td>−3.6</td>
<td>3.2</td>
<td>10.5</td>
<td>242.6</td>
<td>88</td>
<td>80</td>
<td>9</td>
</tr>
<tr>
<td>PGMA 2.0 mol% FdMA</td>
<td>Slab</td>
<td>−3.0</td>
<td>4.4 / 0.7</td>
<td>9.5</td>
<td>248.9</td>
<td>82</td>
<td>75</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Cylinder</td>
<td>−3.8</td>
<td>3.1 / 1.7</td>
<td>11.5</td>
<td>269.5</td>
<td>87</td>
<td>81</td>
<td>7</td>
</tr>
<tr>
<td>Deionized water</td>
<td>---</td>
<td>0.2</td>
<td>3.8</td>
<td>10.6</td>
<td>334.0</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Note: PFMA, poly(fructose methacrylate); PGMA, poly(glucose methacrylate); FdMA, fructose dimethacrylate; T_onset, initial temperature of a thermal event; T_peak, temperature at peak of a thermal event; ΔT, change in temperature; ΔH_w, melting enthalpy of water in the hydrogels.

The hydrolytic stability of the hydrogels was evaluated as a function of the temperature (Fig. 7) and pH (Fig. 8). Since the crosslinker is suitable for hydrolysis, a decrease of the crosslinking density is expected. Therefore, the hydrolytic stability was evaluated by measuring the hydrogel swelling coefficient.

Figure 7 shows that in the buffer solution of pH 7.4 the hydrogels are hydrolytically stable up to 35 °C and, some degree of hydrolysis seems to take place at 45 °C after 24 h, mainly for PGMA 0.5 and 1.0 mol% FdMA and for PFMA 0.5 mol% FdMA.

For slab-shaped hydrogels, the change in the pH of the media from 2 to 10 at 25 °C (Fig. 8) leads to a minor effect on the swelling coefficient indicating that these hydrogels can be hydrolytically stable over this pH range for at least 12 weeks. Hydrogels based on poly(sucrose-1′-diacrylate) crosslinked with sucrose-6,1′-diacrylate, reported by Patil et al., showed similar hydrolytic stability over a pH range from 6 to 7.8 in a period of eight days, but it was completely degraded at pH 9 after six days.

The swelling coefficient of slab shaped PFMA 1.0 mol% FdMA and PGMA 1.0 mol% FdMA immersed in aqueous protease solution at pH 10 is initially around 1000% (Fig. 9). After 240 h, PFMA hydrogel swelling coefficient increased around 2.5 folds, reaching 250%, while for PGMA hydrogel the swelling coefficient increased by around 1.3-fold, indicating a higher degree of degradation for PFMA hydrogel. This is explained by the higher activity of protease towards fructose.13 The biodegradation of hydrogels crosslinked with carbohydrate-based crosslinker by protease and lipase was reported by Patil et al. for poly(acrylamide) and poly(acrylic acid) hydrogels crosslinked with sucrose diacrylate. Wang et al. reported that polymers containing sucrose or lactose moieties undergo enzymatic degradation in the presence of protease, which acts to catalyze ester bond hydrolysis.
Figure 8. Swelling coefficient in aqueous solutions at different pH as a function of time for PFMA and PGMA hydrogels in slab shape. ■ PFMA 0.5 mol% FdMA; ● PFMA 1.0 mol% FdMA; ▲ PFMA 2.0 mol% FdMA; ○ PGMA 0.5 mol% FdMA; ◆ PGMA 1.0 mol% FdMA; △ PFMA 2.0 mol% FdMA.

Figure 9. Swelling coefficient of slab shaped PFMA and PGMA hydrogels in a protease aqueous solution as a function of time. ■ PFMA 1.0 mol% FdMA; ◆ PGMA 1.0 mol% FdMA.

Cell viability essays, expressed as percent cell viability (Cell Viability (%)), are presented in Fig. 10. Cell viability for PFMA and PGMA hydrogels is around 80% (Fig. 10(a)). For similar cell viability essays, Park et al. reported cell viability of around 90% for hydrogels based on β-methylglucoside methacrylate and crosslinked with β-methylglucoside dimethacrylate. Moreover, with the addition of EGDm as a crosslinker in these hydrogels, the cell viability decreases, reaching around 70% at a weight ratio monomer/EGDm of 95:5. These results show that the use of carbohydrate-based crosslinker can endow higher cell viability for biomaterials than using conventional crosslinkers based on fossil resource.

The replacement of Dulbecco’s modified culture medium by culture medium exposed to PFMA hydrogel resulted in an enhancement in cell proliferation compared with control, and also with culture medium exposed of PGMA hydrogel, as can be seen in Fig. 10(b). The differences between the results of PFMA and PGMA are probably related to the higher hydrolysis rate and fructose release rate to the culture medium. Nutrients such as glucose, fructose and fatty acids are reported to stimulate both expression and activation of the key transcription factor, which is reported to increase cell proliferation. Therefore, neither the hydrogels nor their degradation products in culture medium are cytotoxic, making PFMA and PGMA hydrogels suitable materials for biomedical applications.

CONCLUSION

Hydrogels based on fructose and glucose methacrylate crosslinked with different amounts of FdMA resulted in highly hydrophilic macroporous materials that present cell viability higher than 80%, hydrolytic stability over a pH range from 2 to 10 and which degrades in the presence of enzymes. The use of the highly hydrophilic carbohydrate-based crosslinker, such as FdMA, in hydrogel preparation enables the use of high crosslinker concentration, which improves mechanical properties without loss of the water swelling capacity at the same time. This is an important advantage over conventional crosslinkers based on fossil resources. Moreover, hydrogel shape and crosslinking density influences hydrogel morphology, such as pore size and pore
wall thickness, swelling behavior and mechanical resistance. Slab hydrogels hold higher stress under compression–decompression cycles, and present higher resistance to hydrolysis in basic medium due to the thicker pore walls than cylindrical ones, features that can make them promising for wound healing applications. Furthermore, the lower mechanical and hydrolytic resistance of cylindrical hydrogels can be an advantage for drug delivery applications.

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Supporting Information
Supporting information may be found in the online version of this article.

REFERENCES

Figure 10. Cell viability of control (white), PFMA (light gray) and PGMA (gray) hydrogels: (a) MTS assay for 24 h; (b) culture medium exposed to the xerogels.
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