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Synthesis and Photopolymerization of Tween 20 Methacrylate/N-vinyl-2-Pyrrolidone Blends

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ABSTRACT

Poly(oxyethylene 20 sorbitan) monolaurate (Tween® 20) methacrylates were synthesized by coupling methacryloyl chloride (MeOCl) to Tween 20 in the presence of 4-(*N*,*N*-dimethylamino) pyridine, using THF as a solvent, in order to investigate their suitability as precursors for photopolymerizable hydrogels in tissue engineering applications. The degree of substitution could be controlled by adjusting the molar ratio of MeOCl and Tween 20, giving three different monomers: Tween 20 monomethacrylate, Tween 20 dimethacrylate and Tween 20 trimethacrylate. Combined ^{1}H NMR and MALDI-TOF MS confirmed these monomers to be of high purity and to have polydispersities less than 1.3. It was shown that aqueous solutions of the monomers were photoactive, all the methacrylate groups reacting within 30 minutes exposure to a UV light intensity of 145 mW/cm^2. Aqueous Tween 20 trimethacrylate was then combined with N-vinyl-2-pyrrolidone (NVP), giving tough copolymer hydrogels on photopolymerization, whose swelling ratios and swelling rates could be tuned by varying the Tween 20 trimethacrylate content. The use of a flexible spacer with a multifunctional monomer gives a permanent three-dimensional network, whilst maintaining degrees of swelling of between 60 and 85%, with potential for a wide range of biological and non-biological applications.

1. Introduction

Hydrogels produced by photopolymerization have been extensively investigated as biomaterials for applications such as drug delivery carriers, scaffolds, coatings and tissue engineering [1]. Photopolymerization is a method of choice since it involves relatively short reaction times and minimal heating, and it does not require organic solvents [2]. The hydrogel may be prepared from a low viscosity aqueous solution of a monomer, oligomer or macromer via a free radical pathway [3], typically resulting in a highly hydrated structure. A key advantage of photopolymerized hydrogels over chemically or physically crosslinked hydrogels is that their mechanical properties and swelling behavior may be tuned relatively easily by varying the molecular mass and/or the concentration of the precursors. On the other hand, their development is limited by the availability of such precursors and in many cases a high degree of mechanical stability is not required. Three basic strategies have been adopted: (i) use of existing photoactive, water soluble precursors; (ii) addition of photoactive groups to existing non-photoactive hydrophilic precursors; (iii) combination of water-soluble photoinitiators with hydrophilic precursors with limited photoactivity [4]. The third strategy, based on the use of water-soluble photoinitiators, is the most versatile in terms of the range of potential precursors and may be adapted to suit specific wavelengths of UV light, with clear advantages for biomedical applications. Molecules that have been modified for use as precursors for photopolymerizable hydrogels include poly-(ethylene glycol) (PEG) acrylate derivatives [3], PEG methacrylate derivatives [3], poly-(vinyl alcohol) derivatives [5], modified hyaluronic acid [3] and dextran methacrylates [6]. However, these have typically been used in applications where mechanical properties are not determinant, such as drug delivery, soft contact lenses and scaffolds [7]. The present work, on the other hand, focuses on the modification of Tween 20, a bio-inert trifunctional polyoxyethylene derivative of sorbitan monolaurate [8], as a first step towards developing crosslinked hydrated networks with a tailored mechanical response for load-bearing applications. The specific objective is to replace the *nucleus pulposus* in damaged intervertebral discs, an application for which adequate mechanical properties are essential, as discussed in more detail at the end of this article. Tween 20 is a hydro-soluble surfactant used as a detergent and emulsifier in a number of domestic, engineering, and pharmacological applications [9,10], and is distinguished from the other members of the Tween family by the lengths of the polyoxyethylene and the fatty acid ester moieties. It is considered to be an attractive choice for the production of hydrogels for tissue engineering, since it may readily be modified to produce multifunctional photoactive Tween 20 methacrylates, as will be discussed in what follows, and crosslinking via dimethacrylates has been

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Table 1
Compositions of T3/NVP hydrogels.

	T3 [vol%]	I2959 (0.05 wt%) sol. [vol%]	Water [vol%]
T3-4.5	4.5	10	40
T3-8	8	10	40
T3-15	15	10	40

demonstrated previously to be biocompatible, thanks to the low cytotoxicity of the unreacted dimethacrylates [11].

Three, high purity, low polydispersity Tween 20 methacrylate monomers were prepared: Tween 20 monomethacrylate (T1), Tween 20 dimethacrylate (T2) and Tween 20 trimethacrylate (T3). Fourier transform infra-red spectroscopy (FTIR), proton nuclear magnetic resonance (^{1}H NMR) and matrix-assisted laser desorption ionisation time-of-flight mass spectroscopy (MALDI-TOF MS) were used to characterize the monomers and their response to UV irradiation in aqueous solution. The nominally trifunctional T3 monomer was then combined with N-vinyl-2-pyrrolidone (NVP) with the aim of producing three-dimensional hydrogel networks with controlled crosslink densities and hence adjustable degrees of swelling and mechanical properties. NVP was chosen for its biocompatibility [12,13] and also for its tendency to decrease significantly the inhibition of the radical chain polymerization by molecular oxygen [14], which is of particular interest for *in situ* polymerization in biological applications.

2. Experimental section

2.1. Materials

Cell culture tested Tween 20 (MM = 1226 g/mol) was purchased from Sigma-Aldrich, Buchs, dried by azeotropic distillation with benzene and stored in a desiccator. Methacryloyl chloride (MeOCl, 97%), 4-(N,N-dimethylamino)pyridine (DMAP, 99%), α-cyano-4-hydroxycinnamic acid (ChCa) and N-vinyl-2-pyrrolidone (NVP, MM = 111.14 g/mol) were obtained from Sigma-Aldrich Buchs and used as received. Tetrahydrofuran (THF, extra dry, < 50 ppm water) was purchased from Acros Organics, Geel. Other solvents were obtained from Sigma-Aldrich, Buchs. The photoinitiator, Irgacure 2959 (I2959), was obtained from Ciba Specialty Chemicals and used as received.

2.2. Synthesis of Tween 20 mono-, di- and tri-methacrylate

The procedure used to synthesize T3 is described in detail in what follows. Only the proportions of the reagents differ in the procedures used for T1 and T2. Tween 20 (20 g, 0.016 moles) was dissolved in 100 ml of THF in a 250 ml round-bottom flask, to which 6.2 g (0.06 moles) of DMAP was introduced under argon. After cooling to 0 °C in ice, 4.9 ml of MeOCl (0.049 moles) in 30 ml THF was added drop-wise to the mixture over 30 minutes under stirring using a pressure-equalized addition funnel. The flask was covered with aluminum foil and left under stirring overnight. The resulting precipitate was then filtered off, washed with THF and dried in a rotary evaporator at room temperature, avoiding light exposure. Part of the crude product (3 g) was purified by column chromatography (60 g silica, eluant $CHCl_3$/MeOH, 9/1 to 8/2, v/v), yielding 2.2 g of a light yellow viscous liquid, after evaporation of the solvent. The yields obtained after purification were 83% for T1 and T2, and 75% for T3. Thin layer chromatography (TLC) using $CHCl_3$/hexane/methanol (5:5:2 v/v) as the eluent was used to check the purity of each fraction.

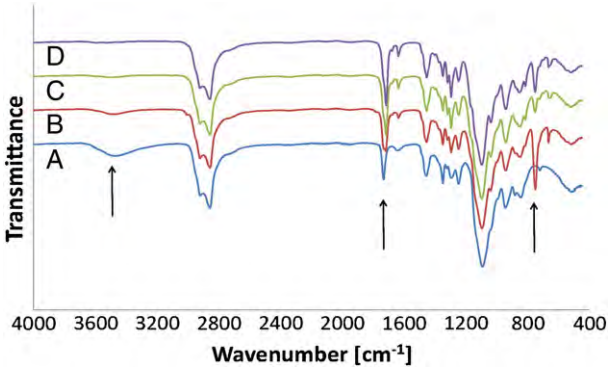


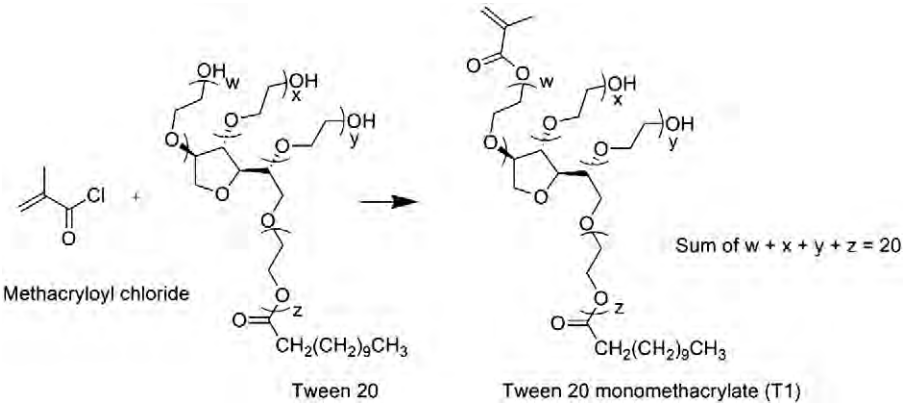
Fig. 1. FTIR spectra from Tween 20 (A) and T1 (B), T2 (C) and T3 (D). Selected peaks are assigned as follows: 3500 cm^{-1} (ν_{O-H}), 2900 cm^{-1} (ν_{C-H}), 1710 cm^{-1} ($\nu_{C=O}$), 1100 cm^{-1} ($\nu_{C-O,\ ester}$), 815 cm^{-1} ($\nu_{C-H,\ C=CH2}$).

2.3. Characterization

FTIR spectra were recorded with a Perkin-Elmer Spectrum One spectrometer, Spectrum Spotlight 300. 64 scans were recorded between 4000 and 400 cm^{-1} at a resolution of 4 cm^{-1} and the peak intensities were determined using Spectrum v 5.0.1 software. ^{1}H-NMR spectra were recorded in $CDCl_3$ (99.8% D, Armar Chemicals) using a Brucker Avance 400 spectrometer, calibrated with tetramethyl silane (TMS). MALDI-ToF MS analyses were performed in triplicate, using a Shimadzu Axima CFRplus MALDI-ToF MS in linear or reflectron mode. The MALDI matrix, α-cyano-4 hydroxycinnamic acid (ChCa), was added to solutions of the Tween 20 methacrylates in THF (1 vol%). 1 μl of each solution was then deposited on a sample plate and dried, prior to acquisition of the corresponding spectrum.

2.4. Crosslinking of Tween 20 methacrylates

T1, T2 or T3 at a concentration of 80 vol% were mixed with 20 vol% aqueous I2959 (0.05 wt% of I2959 in water). Cylindrical samples of 5 mm in height and 6 mm in diameter were photopolymerized for 30



Scheme 1. Reaction of Tween 20 with MeOCl to give T1.

minutes with a UV light source (270 nm to 370 nm, 145 mW/cm^2). After photopolymerization, the relative decrease in the heights of the FTIR absorption peaks characteristic of methacrylates at 1710 cm^{-1} and 815 cm^{-1}, was used to estimate the conversion of the methacrylate groups.

2.5. Crosslinking and characterization of T3/NVP

Cylindrical samples of 5 mm in height and 10 mm in diameter of the T3/NVP-based hydrogels were prepared by photopolymerization of T3/NVP precursor solutions in the presence of aqueous I2959 (0.05 wt% in water) with the compositions given in Table 1. The cure conditions were as described in the previous section. The swelling behavior was monitored gravimetrically by measuring the weight gain with time of immersion in phosphate buffered saline (PBS) at room temperature. The samples were wiped off and weighed every 15 minutes, until equilibrium was reached. The equilibrium swelling ratio *SR* was calculated from:

$$SR = W_s/W_d = (W_w - W_d)/W_d \quad (1)$$

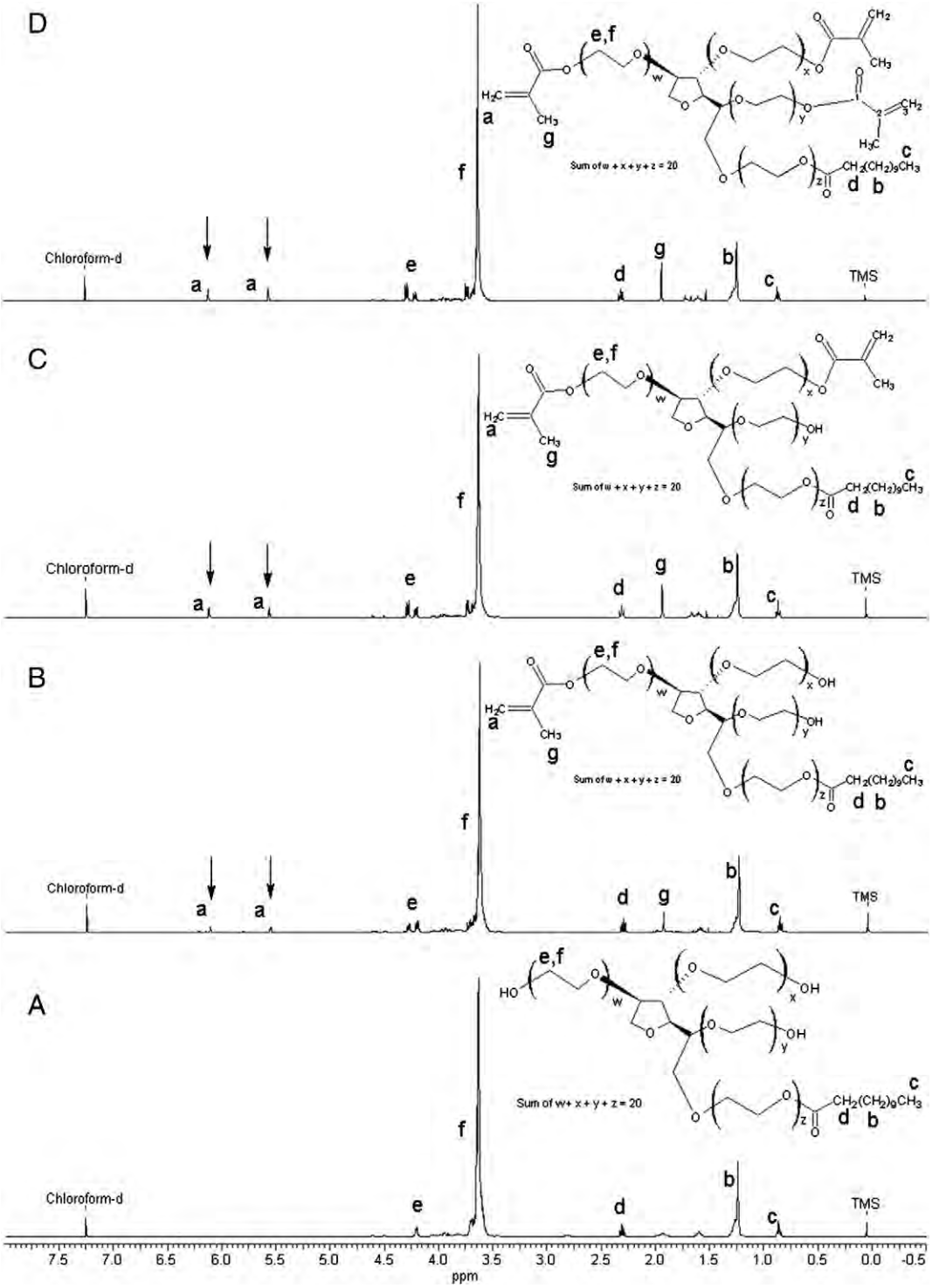


Fig. 2. ^{1}H NMR spectra from Tween 20 (A), T1 (B), T2 (C) and T3 (D).

Table 2
Value of integrals of methacrylate peaks from ^{1}H NMR.

	DS theoretical	DS δ 5.6 ppm	DS δ 6.1 ppm
T1	1	1.01	0.96
T2	2	2.08	2.01
T3	3	3.07	3.17

where W_s is the weight of PBS in the swollen hydrogel at equilibrium, W_w is the weight of the wet sample and W_d is the weight of the hydrogel at time 0. The transport mechanism controlling PBS sorption and indicating the relative importance of diffusion and relaxation is typically determined by fitting swelling data (for short times or $SR_t/SR_\infty \leq 0.6$) to the empirical expression given in Eq. (2), where SR_t is the swelling ratio at time t, SR_∞ is the swelling ratio at equilibrium, and K is a rate constant characteristic of the polymer-solvent system [15].

$$SR_t/SR_\infty = Kt^{n'} \tag{2}$$

For the cylindrical geometry, a value of $n' \leq 0.45$ indicates a swelling rate controlled by Fickian diffusion, a value of $n' = 1$ implies relaxation-controlled transport and intermediate values define anomalous transport [15]. For a Fickian transport mechanism, the rate of approach to swelling equilibrium may be characterized by the diffusion coefficient of the solvent, D, such that

$$K = 4\left(D/\pi l^2\right)^{n'} \tag{3}$$

where l is the initial thickness of the sample [16]. The values of K and n' were calculated from the slopes and intercepts of plots of $\log(SR_t/SR_\infty)$ vs $\log t$, obtained by linear regression [15].

3. Results and discussion

3.1. Synthesis and characterization of T1, T2 and T3

The general synthetic route for coupling a vinyl moiety with a hydroxylated polymer was first described by Hennink [17]. The Tween 20 hydroxyl endgroups react with the methacryloyl chloride moieties in the presence of a base (DMAP), which acts as a catalyst, to form the Tween methacrylates. Under basic reaction conditions, the hydroxyl groups of Tween 20 are polarized and react subsequently with the less hindered methylene carbon of the epoxy group of MeOCl, according to Scheme 1.

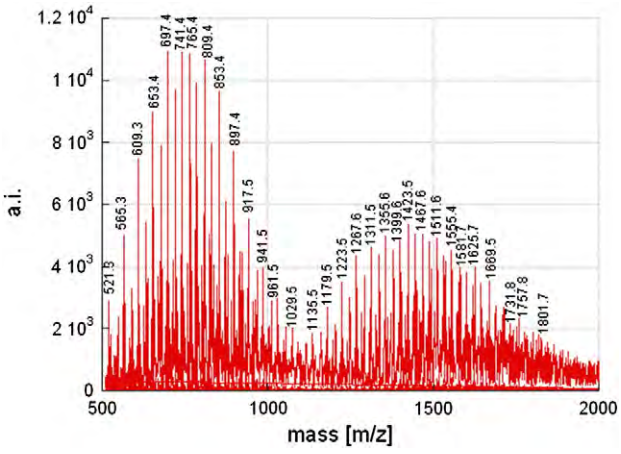


Fig. 4. MALDI-ToF mass spectrum from T1.

FTIR spectra for Tween 20 and the different monomers are shown in Fig. 1. With the exception of the –OH absorption at around 3500 cm^{-1}, the C=O absorption at around 1700 cm^{-1} and the peak at 813 cm^{-1} associated with the double bond of the methacrylate moiety, the FTIR spectra were essentially identical, suggesting no modification to the monomer backbone. On the other hand, the decrease in the strength of the –OH absorption, the increase in the C=O absorption and the appearance of the peak at 813 cm^{-1} were consistent with the conversion of the –OH groups into methacrylate groups, the –OH absorption disappearing altogether in the case of T3. Quantitative analysis was nevertheless difficult owing to superposition of the C=O absorption associated with the methacrylate groups onto that associated with other C=O groups present in the Tween 20 backbone.

^{1}H NMR spectra for Tween 20 and the different monomers are shown in Fig. 2. Tween 20 is characterized by peaks at δ0.8 ppm, δ1.2 ppm, δ1.6 ppm, δ3.65 ppm and δ4.2 ppm. The peak at δ3.65 ppm is characteristic of the PEG structure and represents the inner protons, whereas that at δ4.2 ppm is representative of the protons adjacent to the end groups. These peaks were present in all the spectra, again indicating the backbone of the molecule to remain unchanged after the reaction. The lack of additional peaks other than those associated with the methacrylate moieties in the T1, T2 and T3 spectra suggests that unreacted compounds were quantitatively removed. Peaks at δ5.8 and 6.2 ppm are reported for the methylene protons of methacrylate groups [3]. In the present case, peaks at δ5.6 and 6.1 ppm were observed in T1, T2 and T3, and the associated intensities increased with the degree of methacrylation. The peak at δ4.2 ppm corresponded to the protons adjacent to the hydroxyl groups in the Tween 20 molecule. After

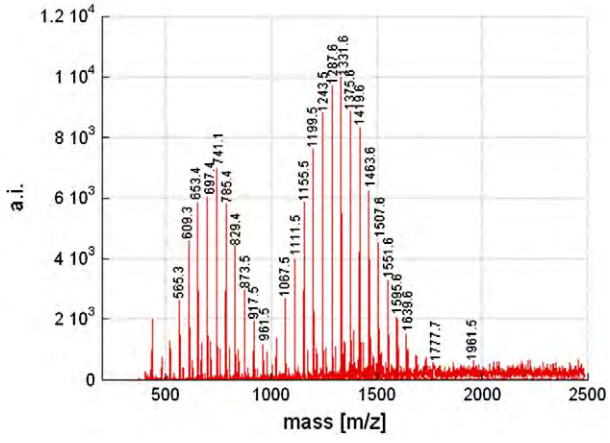


Fig. 3. MALDI-ToF mass spectrum from Tween 20.

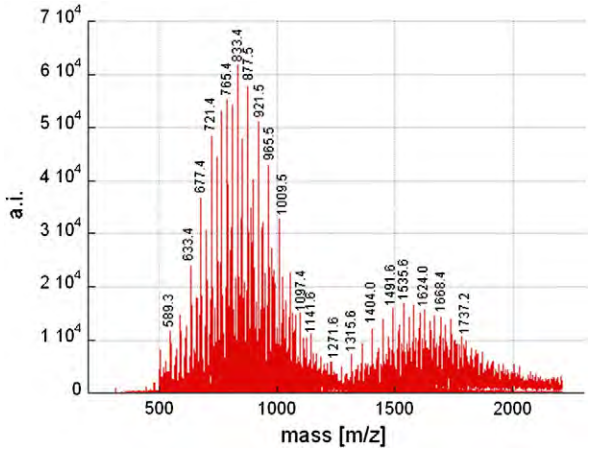


Fig. 5. MALDI-ToF mass spectrum from T2.

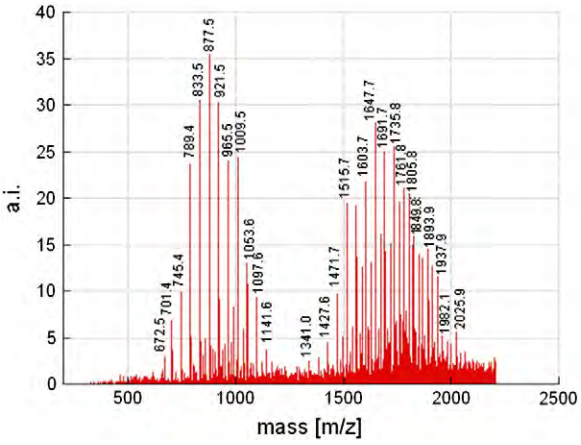


Fig. 6. MALDI-ToF mass spectrum from T3.

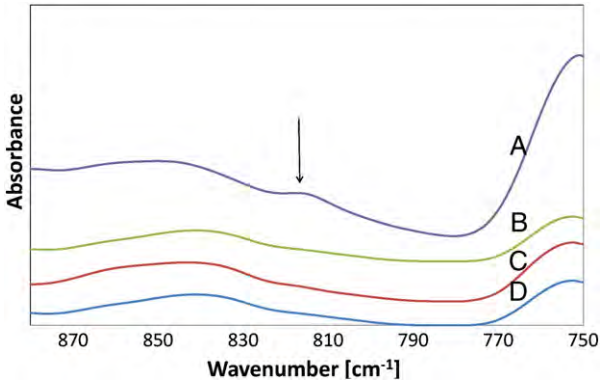


Fig. 7. Expanded FTIR spectra from unpolymerized T1 (A), polymerized T1 (B), polymerized T2 (C) and polymerized T3 (D).

methacrylation, an additional peak was observed at δ4.3 ppm, due to protons adjacent to the newly added methacrylate moieties. Unreacted hydroxyl groups could not be distinguished in the spectra owing to overlap with the inner proton peak at δ3.65 ppm. In order to quantify the degree of methacrylation, the peak at δ1.2 ppm, which corresponds to 18 of the olefinic protons, was used on as reference. The relative intensities of the peaks, given in Table 2, confirmed the target conversions to have been achieved to within the accuracy of the measurement (experimental error<5%). Thus, whereas full conversion of the –OH groups was obtained in T3, consistent with the FTIR spectra, one and two –OH groups per molecule were converted in T1 and T2 respectively.

The number average molar mass, M_n, may be estimated from the ^{1}H NMR spectra by comparing the methacrylate proton peak intensity (g) to the reference peak (b) (Fig. 2). Since the unreacted hydroxyl groups of the Tween 20 cannot be distinguished by ^{1}H NMR owing to overlap with the inner Tween 20 proton peaks, the molecular mass calculations must assume stoichiometric conversion, i.e. exact correspondence between the number of reacted –OH groups and the number of methacrylate groups initially present in the reaction mixture, which may not necessarily be the case, depending on the degree of steric hindrance and the excess of MeOCl.

MALDI-ToF was also used to provide an independent measure of M_n and M_w. Ayorinde et al. [18] tested a commercial formulation of Tween 20 purchased from Sigma Aldrich and very similar MALDI-ToF spectra were obtained in the present work for the same material, as shown in Fig. 3, suggesting the technique to provide consistent results in the present case. The double peaks in the spectra reflected the different oligomers present in the formulation, and the ions within each distribution were 44 Da apart, which corresponds to the ethoxy repeat unit present in the Tween 20 molecule (Figs. 4, 5 and 6). The molar masses determined for Tween 20, T1, T2 and T3 by ^{1}H NMR and MALDI-ToF are summarized in Table 3. In each case, M_n obtained by the two techniques were close, and any discrepancies could be attributed to fragmentation of the Tween 20 and associated monomers during laser desorption, as observed in previous measurements on PEG dimethacrylate molecules [3]. As the degree of methacrylation increased, the polydispersity, M_w/M_n (PDI), decreased significantly. This effect was attributed to steric hindrance; as the molecular chains become bulkier steric hindrance limits the formation of many of the molecular species and the associated molar masses present for low degrees of methacrylation.

Table 3
Molar masses (g/mol) from Tween 20 methacrylates obtained by ^{1}H NMR and MALDI-ToF mass spectroscopy.

	M_n (NMR)	M_n (MALDI)	M_w (MALDI)	PDI
T1	1271	1275	1526	1.20
T2	1408	1302	1475	1.13
T3	1560	1831	1870	1.02

3.2. Polymerization

The methacrylate groups in T1, T2 and T3 can react with UV light to form highly reactive radical species. Fig. 7 shows details of the FTIR spectra for the photopolymerized methacrylates in the range corresponding to the C=C absorption peak at 813 cm^{-1} The C=C absorption peaks were no longer visible after photopolymerization, indicating high conversion of the double bonds. On exposure to UV light, T1 showed a significant increase in viscosity and T2 and T3 formed stable hydrogels. However, both of these latter where extremely brittle owing to the limited deformability of the networks and were therefore not considered for further testing.

3.3. T3/NVP hydrogels

To improve the deformability and hence the toughness of the photopolymerized gel systems, it was decided to use T3 as a crosslinker in combination with a flexible linear spacer, NVP. The resulting hydrogels were macroscopically homogeneous and highly deformable at all T3 concentrations. They were initially relatively transparent at low T3 contents, but became more opaque as the T3 content was increased, and totally opaque at swelling equilibrium, presumably due to inhomogeneities formed at the macromolecular

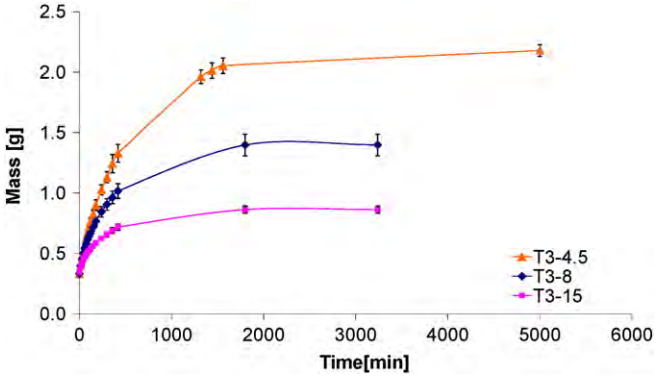


Fig. 8. Mass water uptake of various T3/NVP with T3 contents of 4.5, 8 and 15 vol% (Table 1).

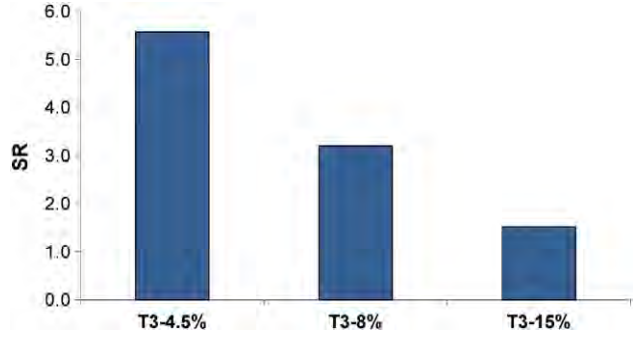


Fig. 9. Swelling ratio of various T3/NVP hydrogels with T3 contents of 4.5, 8 and 15 vol% (Table 1).

Table 4
n' as a function of T3 concentration.

	n'
T3-4.5	0.38
T3-8	0.29
T3-15	0.19

level during polymerization [19]. All T3/NVP hydrogels showed similar swelling behavior, with rapid swelling at short times (300 to 500 minutes) and more gradual swelling at intermediate times, the swelling ratio tending asymptotically to its equilibrium value, *SR*, at the longest times. Representative data are shown in Fig. 8. The hydrogel composition had a significant influence on the degree of swelling, with $SR = 5.6$ for T3-4.5 but $SR = 1.5$ for T3-15, as shown in Fig. 9. This trend is consistent with an increase in crosslink density in the presence of the branched T3 molecules. The rate-determining step in the swelling process is the stress relaxation of the polymers chains in response to the osmotic pressure. The decay in the rate of swelling with time is therefore expected to be more pronounced in systems in which the relaxation of the chains is slower, i.e. in which a more highly crosslinked network is formed. In the present case, *n'* was less than 0.45 at all T3 concentrations and decreased with increasing T3 concentration, as shown in Table 4, indicating Fickian diffusion of the PBS to be dependent on the T3 concentration [15]. Fickian behavior has been reported previously for hydroxyethyl methacrylate-based copolymeric hydrogels [20], whereas non-Fickian behavior is observed in NVP-based copolymer hydrogels [21]. Using T3 as crosslinker together with NVP changes the diffusion mechanism to Fickian, indicating the determining role of T3 in the construction of the polymer network. As shown in Fig. 10, *D* decreased with increasing T3 concentration, again

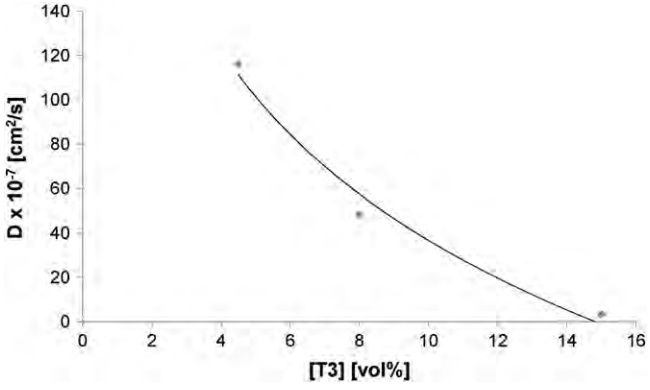


Fig. 10. Diffusion coefficient *D* in T3/NVP hydrogels as a function of T3 content.

consistent with an increase in crosslink density, which is expected to inhibit penetration of the network by the solvent.

4. Conclusions

An efficient method has been developed for the synthesis of Tween 20 monomethacrylate (T1), dimethacrylate (T2) and trimethacrylate (T3). A combination of ^{1}H NMR and MALDI-ToF mass spectroscopy was shown to provide detailed information on the extent of the reaction, the molar mass and the molar mass distribution, demonstrating a high degree of conversion and low impurity contents in each case. Photopolymerization of aqueous T1, T2 or T3 with Irgacure 2959 was shown to give close to complete conversion of the methacrylates after 30 minutes UV exposure. The resulting hydrogels were extremely brittle and therefore not considered for further testing. However, by combining T3 with a polymeric spacer, NVP, it was possible to obtain highly deformable hydrogels with correspondingly high swelling capacities, which could be tailored by varying the T3 content. The swelling kinetics were shown to be controlled by Fickian diffusion of water into the network and the diffusion rate was reduced as the amount of T3 was increased, reflecting a higher degree of crosslinking.

As stated in the introduction, one potential application for these materials is the replacement of the *nucleus pulposus* (NP), the inner core of the intervertebral disc, which is essentially a hydrogel. The water content of the NP ranges from 65 to 90% [22], which is similar to the values of 60 to 85% obtained in the present case and is crucial for the overall mechanical response of disc [23,24]. As described in detail elsewhere [25–27], the present T3/NVP hydrogels have been demonstrated to be biocompatible, and compression tests on hydrated specimens indicate viscoelastic behavior comparable with that of human *nucleus pulposus*, confirming this system to be an excellent candidate for intervertebral disc repair.

Acknowledgement

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References

[1] N. Peppas, Hydrogels in medicine and pharmacy, CRC Press, 1986.
[2] J.H. Lee, D.G. Bucknall, J. Polym. Sci., Part B: Polym. Phys. 46 (2008) 1450–1462.
[3] S. Lin-Gibson, S. Bencherif, J.A. Cooper, S.J. Wetzel, J.M. Antonucci, B.M. Vogel, F. Horkay, N.R. Washburn, Biomacromolecules 5 (2004) 1280–1287.
[4] J.P. Fisher, D. Dean, P.S. Engel, A.G. Mikos, Annu. Rev. Mater. Sci. 31 (2001) 171–181.
[5] P.J. Martens, S.J. Bryant, K.S. Anseth, Biomacromolecules 4 (2003) 283–292.
[6] W.N.E. van Dijk-Wolthuis, O. Franssen, H. Talsma, M.J. Van Steenbergen, J.J. Kettenes-van den Bosch, W.E. Hennink, Macromolecules 28 (1995) 6317–6322.
[7] J. Kopecek, J. Polym. Sci., Part A: Polym. Chem. 47 (2009) 5929–5946.
[8] B. Balakrishnan, N.R. James, A. Jayakrishnan, Polym. Int. 54 (2005) 1304–1309.
[9] A. Atwood, A.T. Florence, Surfactant systems: their chemistry, pharmacy and biology, Chapman and Hall, London, 1983.
[10] M.R. Porter, Handbook of Surfactants, 2nd ed. Blackie Academic, London, 1994.
[11] M. Prasitsilp, T. Siriwittayakorn, R. Molloy, N. Suebsanit, P. Siriwittayakorn, S. Veeranondha, J. Mater. Sci. Mater. Med. 14 (2003) 595–600.
[12] L.C. Lopérgolo, A.B. Lugão, L.H. Catalaini, J. Appl. Polym. Sci. 86 (2002) 662–666.
[13] J. Thomas, A. Lowman, M. Marcolongo, J. Biomed. Mater. Res. A 67 (2003) 1329–1337.
[14] T.J. White, W.B. Liechty, C.A. Guymon, J. Polym. Sci., Part A: Polym. Chem. 45 (2007) 4062–4073.
[15] P.L. Ritger, N.A. Peppas, J. Control. Release 5 (1987) 23–36.
[16] B.G. Kabra, S.H. Gehrke, S.T. Hwang, W.A. Ritschel, J. Appl. Polym. Sci. 42 (1991) 2409–2416.
[17] W.E. Hennink, C.F. van Nostrum, Adv. Drug Deliv. Rev. 54 (2002) 13–36.
[18] F.O. Ayorinde, S.V. Gelain, J.H. Johnson Jr., L.W. Wan, Rapid Commun. Mass Spectrom. 14 (2000) 2116–2124.
[19] M. Shibayama, Macromol. Chem. Phys. 199 (1998) 1–30.
[20] D.J.T. Hill, M.C.H. Lim, A.K. Whittaker, Polym. Int. 48 (1999) 1046–1052.
[21] M. Sen, N. Pekel, O. Guven, Makromol. Chem. 257 (1998) 1–6.
[22] J. Antoniou, T. Steffen, F. Nelson, N. Winterbottom, A.P. Hollander, R.A. Poole, M. Aebi, M. Alini, J. Clin. Invest. 98 (1996) 996–1003.

[23] Q.B. Bao, G.M. McCullen, P.A. Higham, J.H. Dumbleton, H.A. Yuan, Biomaterials 17 (1996) 1157–1167.
[24] B. Vernon-Roberts, in: M.I. Jayson (Ed.), The lumbar spine and back pain, Churchill Livingstone, London, 1992, pp. 17–41.
[25] A.C. Borges, P.E. Bourban, D.P. Pioletti, J.A.E. Manson, Compos. Sci. Technol. 70 (2010) 1847–1853.
[26] A.C. Borges, C. Eyholzer, F. Duc, P.E. Bourban, P. Tingaut, T. Zimmermann, D.P. Pioletti, J.A.E. Manson, Acta Biomater. 7 (2011) 3412–3421.
[27] C. Eyholzer, A.C. Borges, F. Duc, P.E. Bourban, P. Tingaut, T. Zimmermann, J.A.E. Manson, K. Oksman, Biomacromolecules 12 (2011) 1419–1427.