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Synthesis and characterisation of chitosan-*graft*-poly(OEGMA) copolymers prepared by ATRP

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ABSTRACT

Two synthetic routes, "grafting-from" and "grafting-to" the chitosan backbone, were investigated to prepare chitosan-*graft*-poly(OEGMA) copolymers by ATRP. The copolymers were characterised by ^{1}H NMR and FT-IR spectroscopy, elemental analyses and GPC. Estimates of the degree of grafting were quantitatively found from integration of the ^{1}H NMR spectra. The GPC data indicated that the hydrodynamic volumes of the polymers were significantly altered by the incorporation of poly(OEGMA). A comparison of the two synthetic methods showed differences in the ratio of synthetic and natural polymers present in the resulting combs. The "grafting-to" synthetic route was preferable as the polymers were of higher purity and more defined.

1. Introduction

Chitin is a biopolymer typically extracted from crab and shrimp shells and is the second most abundant polysaccharide after cellulose. This interesting polysaccharide can be deacetylated to give chitosan with an amine group in the C2 position. Interest in chitosan has grown in recent years as its favourable properties have become more understood. Chitosan is soluble, haemostatic, biodegradable, biologically inert under most physiological conditions and non-toxic, which makes it suitable for use in food, cosmetics and medical applications. It has been approved for human use by the FDA (Wedmore, McManus, Pusateri, & Holcomb John, 2006) and the European Pharmacopeia. Recent interest has turned to enhancing the properties of the parent polymer by modification to form new derivatives for specific applications (Kurita, 2001; Munro, Hanton, Robinson, & Simpson, 2008; Werle & Bernkop-Schnürch, 2008).

Graft copolymers are an emerging group of new polymers which consist of two types of polymers in one molecule. These materials can have properties which are different from both of the parent polymers. Addition of other polymers to chitin or chitosan can give derivatives with different properties (such as improved solubility) which are targeted to specific applications. One particular group of copolymers are chitosan-*graft*-poly(ethylene glycol) polymers. These semi-synthetic materials, with the natural polysaccharide as the backbone and poly(ethylene glycol) (PEG) chains as the side chains, are of interest due to the inert behaviour of both polymers in physiological systems (Lebouc, Dez, Desbrières, Picton, & Madec, 2005; Zohuriaan-Mehr, 2005). PEG, like chitosan, shows promise for drug delivery systems and as scaffolding materials and is also widely utilised as a suitable polymeric material in biological applications due to its non-toxicity and biocompatibility (Huang, Shen, & Fang, 2005). This polymer is one of only a few synthetic polymers that have been approved for internal use including in food, cosmetics and pharmaceuticals (Gorochovceva & Makuška, 2004). The resulting semi-synthetic materials are therefore expected to combine the favourable properties of both polymers and show great potential in biological systems. An increased affinity for organic solvents has also been proposed (Kurita, Amemiya, Mori, & Nishiyama, 1999) for this group of hybrid materials.

One widely used synthetic technique which has been developed for chitosan-*graft*-PEG copolymers is a "protection-graft-deprotection" method where the amine groups of chitosan are initially reacted with phthalic acid to form *N*-phthaloyl chitosan (Gorochovceva & Makuška, 2004; Huang et al., 2005; Lebouc et al., 2005; Liu, Li, Fang, & Guo, 2006). PEG is grafted onto this derivative and the resulting materials are deprotected with hydrazine. Both reactions may not result in complete conversion however, with significant amounts of the phthaloyl groups present in the final polymer even after additional treatment with hydrazine monohydrate (Lebouc et al., 2005; Liu et al., 2006). Reductive alkylation has also been used to prepare chitosan-*graft*-PEG copolymers (Kulkarni et al., 2006; Kurita et al., 1999). This "grafting-to" method involves reactions of an aldehyde with an amine group of the chitosan to form a Schiff base, which is reduced to an alkyl group. The PEG chain is of a specified length and can be added as an aldehyde (Kurita et al., 1999) or reacted with the Schiff bases present in the chain (Kulkarni et al., 2006).

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An alternative method involves preparing grafted chitosan copolymers by a living polymerisation technique, such as atom transfer radical polymerisation (ATRP) (El Tahlawy & Hudson, 2003; Munro, Hanton, Moratti, & Robinson, 2007; Zohuriaan-Mehr, 2005). The use of "living" polymerisation techniques has led to more control of the formation of graft copolymers giving well-defined polymer structures. The relative infancy of this research area means that more research into grafted copolymers prepared by these "living" techniques will lead to a greater understanding of structure–property relationships (Jenkins & Hudson, 2001). ATRP involves the exchange of a halide between the propagating chain and a suitable catalyst which provides control of the molecular weights of the resulting polymers. ATRP is possible under a wide range of reaction conditions including in aqueous solvents and in the presence of acid or base, with a number of different catalysts. Chitosan can therefore be altered under homogeneous reaction conditions.

Herein, we report the synthesis and characterisation of a series of initiators and polymers which were subsequently used to prepare chitosan-*graft*-poly(oligoethylene glycol methacrylate) copolymers by two synthetic routes: "grafting-from" (Fig. 1) and "grafting-to" (Fig. 2). Poly(oligoethylene glycol methacrylate) (poly(OEGMA)) was used in this work as it is soluble and biologically inert leading to graft copolymers which are potentially suitable for biomaterials. ATRP was used to prepare poly(OEGMA) as this technique is suitable over a range of reaction conditions including in acidic aqueous solutions.

2. Experimental

2.1. Materials

Chitin was extracted from squid pens which were kindly donated by Otakou Fisheries, Dunedin, New Zealand. The monomer OEGMA (M_n = 475 g mol^{-1}, Sigma–Aldrich) was filtered through basic alumina and purged with nitrogen or argon gas prior to use. All the reactions were carried out under an inert atmosphere of either nitrogen or argon and the solvents used in the polymerisations were degassed with argon. The dextran standards and copper(I) bromide (CuBr) (Sigma–Aldrich) were stored under argon. The prepared initiators **2a** and **2b** were analysed by ^{1}H NMR spectroscopy and were purified before use if required. Ethyl acetate was distilled over CaH_2 before use. All other reagents were purchased from Sigma–Aldrich and used as received.

2.2. Characterisation

IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer using KBr discs with 16 scans and 4 cm^{-1} resolution. HRMS was performed using a Bruker micrOTOF$_Q$ in positive ESI mode with samples dissolved in MeOH. X-ray diffraction data were collected on a Bruker APEX II CCD diffractometer, with graphite monochromated Mo-Kα (λ = 0.71073 Å) radiation. Intensities were corrected for Lorentz-polarization effect (Otwinowski & Minor, 1997; SAINT, 1996) and a multiscan absorption correction (Sheldrick, 1996) was applied. The structures were solved by direct methods with SIR-97 (Altomare et al., 1999) and refined on F^2 using all data by full-matrix least-squares procedures in SHELXL 97 (Sheldrick, 2008). All calculations were performed using the WinGX interface (Farrugia, 1999). Crystallographic data for the structural analysis of **2a** and **2b** have been deposited with the Cambridge Crystallographic Data Centre. CCDC reference numbers: 710488 and 710489.

Microanalyses were performed by the Campbell Microanalytical Laboratory, University of Otago. The molecular formulae of the various polymeric sugar units (*N*-acetyl-glucosamine, glucosamine, *N*-(α-bromopropionyl)-glucosamine and *N*-(α-bromo-α-methylpropionyl)-glucosamine), poly(OEGMA) and water were entered into a Microsoft Excel spreadsheet. The percentage of each component was varied to give an estimate of the percentages of C, H, N and Br. These calculated values were compared to the experimentally determined mass percentage values and adjusted to give the lowest sum of squares for each product. ^{1}H NMR spectroscopy was performed at 25 °C on a Varian Unity Inova 300 MHz or

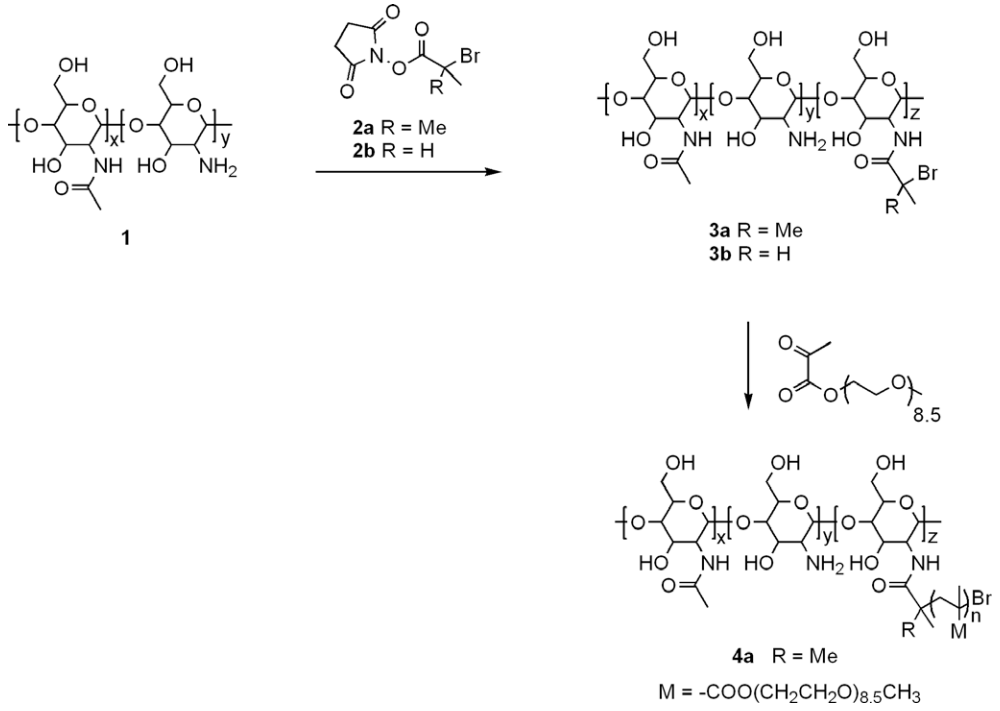

Fig. 1. The "grafting-from" synthetic route used to prepare chitosan-*graft*-poly(OEGMA) copolymers.

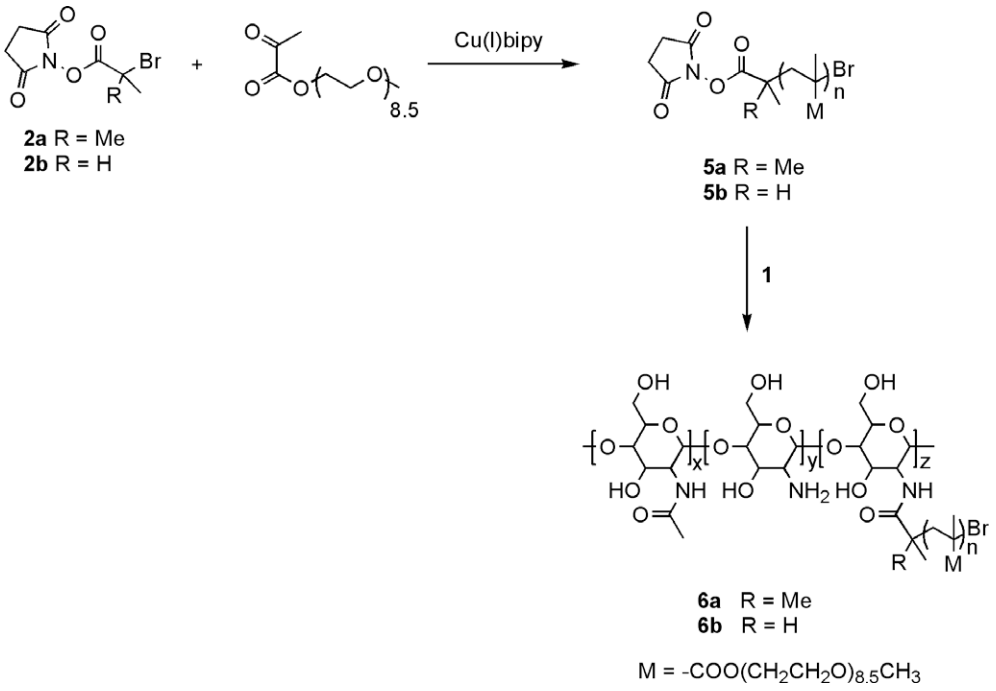

Fig. 2. The "grafting-to" synthetic route used to prepare chitosan-*graft*-poly(OEGMA) **6** from chitosan **1** and poly(OEGMA) **5**.

500 MHz spectrometer in $CDCl_3$, D_2O or d_4-AcOD/D_2O referenced to trimethylsilyl-2,2,3,3-tetradeuteropropionic acid (d_4-TSP). The spectra of the initiators **2a** and **2b** were acquired in $CDCl_3$ with 32 scans. Aliquots from the polymerisation reactions were diluted in D_2O, left in air and the ^{1}H NMR spectra were obtained with 128 scans at 25 °C without spinning of the samples. Baseline corrections were applied to the acquired spectra. The integrated areas for each peak were measured over two times the full-width half-maximum (FWHM) of each chosen peak centred on the peak centre as this area was expected to measure 95% of the peak intensity (Harris, 2003). The monomer conversions in the ATRP reactions of OEGMA were monitored by comparison of the intensities of the methylene peaks at 4.39 and 4.24 ppm in the monomer and polymer, respectively. The amount of synthetic polymer in the chitosan comb polymers was determined by the ratio of the methyl peak ($ROCH_3$) from the synthetic polymer poly(OEGMA) at 3.44 ppm to the H2 chitosan peak at 3.18 ppm.

The aliquots from the polymerisation reactions were purified by dialysis to remove the copper ions, unreacted monomer and D_2O before measurement by GPC. The MW distributions were determined with a PL-GPC 50 integrated system using a Shodex OHpak SB-805 HQ column with Shodex OHpak guard column set at 30 °C and an RI detector. The solvent 0.3 M AcOH/0.2 M AcONa(aq) was used at a flow rate of 1 mm min^{-1}. Aliquots (20 μL) of each filtered 1 mg mL^{-1} solution (0.45 μm filters) of the dextran standards and the samples were measured in duplicate. The data were analysed with Cirrus GPC software version 3.1.

2.3. Extraction of chitin

Dried cut-up squid pens (50.81 g) were stirred in 1.0 M NaOH(aq) solution (1.0 L) overnight. The extracted chitin was washed until neutral, excess moisture squeezed out and the chitin dried overnight at 50 °C. The dry chitin was ground up in a grain mill with a 0.8 mm mesh to give 14.12 g of a white fluffy powder. Calculated for $(Chitin)_{0.88}(Chitosan)_{0.12}\cdot 1.0H_2O$: C, 43.1; H, 6.9; N, 6.5. Found: C, 43.1; H, 6.8; N, 6.4. IR ($\tilde{\nu}$, cm^{-1}): 3416 (OH str) 2926 (CH str) 1638 (NH bend) 1618 (NH bend) 1559 (amide III) 1377, 1321, 1154 (pyranose) 1114 (pyranose) 1070 (pyranose) 1032 (pyranose) 604, 477.

2.4. Deacetylation of chitin to chitosan *1*

Chitin (12% DD, 14.00 g, 64.76 mmol) was stirred in 10 M NaOH(aq) solution (280 mL) at 70 °C for 2 h. The product was washed with water until neutral and dried overnight at 50 °C to give 7.18 g of a cream solid. The treatment was repeated to give 5.61 g of a cream solid **1**. Calculated for $(Chitin)_{0.20}(Chitosan)_{0.80}\cdot 0.8H_2O$: C, 41.8; H, 7.1; N, 7.6. Found: C, 41.8; H, 7.1; N, 7.6. IR ($\tilde{\nu}$, cm^{-1}): 3419 (OH str) 2923 (CH str) 2854 (CH str) 2150, 1638 (NH bend) 1617 (NH bend) 1418, 1384, 1323, 1155 (pyranose) 1074 (pyranose) 601, 466. ^{1}H NMR (δ, ppm, d_4-AcOD/D_2O): 3.17 (s, chitosan **H**2) 3.71 and 3.90 (2× s, chitosan **H**3, **H**4, **H**5, **H**6) 4.78 (s, chitosan **H**1). GPC (g mol^{-1}): M_p = >3,000,000; M_w = >3,000,000; M_n = 838,000.

2.5. (2,5-Oxo-1-pyrrolidyl)oxy-2-bromo-2-methylpropionate *2a*

The reagents *N*-hydroxysuccinimide (NHS) (1.15 g, 10.0 mmol) and Et_3N (2.8 mL, 20 mmol) were dissolved in CH_2Cl_2 (200 mL) and the solution cooled in ice. The reagent α-bromoisobutyryl bromide (BIBB) (1.36 mL, 11.0 mmol) was slowly added and the solution was stirred in ice for 1 h then at ambient temperature for 2 h. The reaction mixture was poured into distilled water (150 mL) and the aqueous layer extracted with diethyl ether (3 × 20 mL). The combined organic solution was washed sequentially with saturated $NaHCO_3$(aq) solution (3 × 20 mL), dilute HCl(aq) solution (pH 5) (3 × 20 mL) and saturated $NaHCO_3$(aq) solution (3 × 20 mL). The organic layer was dried over $MgSO_4$ and reduced in volume to give 2.37 g (90.0%) of a pale yellow solid **2a**. Calculated for $C_8H_{10}NO_4Br$: C, 36.4; H, 3.8; N, 5.3; Br, 30.3. Found: C, 36.4; H, 3.8; N, 5.1; Br, 30.2. IR ($\tilde{\nu}$, cm^{-1}): 2986 (CH str) 2945 (CH str) 1808 (C═O str) 1776 (C═O str) 1730 (C═O str) 1466 (CH bend) 1456 (CH bend) 1371 (CH bend) 1255 (CO str) 1204 (CH bend) 1075 (CO str) 924 (CO str) 734 (CH rock). ^{1}H NMR (δ, ppm, $CDCl_3$): 2.07 (s, 6H, C**H**$_3$) 2.85 (s, 4H, **H**$_{cycl}$). ^{13}C NMR (δ, ppm, $CDCl_3$): 25.61 (**C**$_{cycl}$) 30.72 (C(**C**H$_3$)$_2$Br) 51.12

(**C**$(CH_3)_2$Br) 167.48 (C=O) 168.47 (**C**$_{cycl}$=O). HRMS (+ESI): *m/z* calculated for $[M+Na]^+$: 285.9685. Found: 285.9676. Crystallographic quality crystals were prepared by slow evaporation of an acetonitrile solution. *Crystal data* – $C_8H_{10}NO_4Br$, *M* 264.08, orthorhombic, space group $P2_12_12_1$, a = 9.4280(10), b = 12.2202(13), c = 17.8689(19) Å, V = 2056.7(6) Å^3, Z = 8, $F(000)$ = 1056, μ(Mo-Kα) = 0.091 cm^{-1}, T = 90(2) K, 2984 unique reflections. Refinement of 257 parameters converged at final R_1 = 0.037, wR_2(all data) = 0.079.

2.6. (2,5-Oxo-1-pyrrolidyl)oxy-2-bromopropionate **2b**

The reagents NHS (2.76 g, 24.0 mmol) and 2-bromopropionic acid (BPA) (1.80 mL, 20.0 mmol) were dissolved in CH_2Cl_2 (440 mL) in an ice bath. A solution of *N,N′*-dicyclohexylcarbodiimide (DCC) (4.13 g, 20.0 mmol) in CH_2Cl_2 (10.0 mL) was added dropwise. The clear solution was stirred for 16 h from 0 °C to ambient temperature. The opaque white suspension was filtered and the filtrate was reduced under vacuum to give a white solid to which diethyl ether was added. The resulting suspension was filtered and the filtrate reduced to dryness. The solid was dissolved in CH_2Cl_2 and the solution was washed with saturated Na_2CO_3(aq) solution (2 × 10 mL) and distilled water (2 × 10 mL). The solution was reduced in volume and the resulting solid was dried under vacuum to give 3.87 g (77.3%) of a white solid **2b**. Calculated for $C_7H_8NO_4Br$: C, 33.6; H, 3.2; N, 5.6; Br, 32.0. Found: C, 34.2; H, 3.3; N, 5.6; Br, 31.3. ^{1}H NMR (δ, ppm, $CDCl_3$): 1.96 (d, 3H (J = 6.9 Hz) CH(C**H**$_3$)Br) 2.86 (s, 4H, **H**$_{cycl}$) 4.61 (q, 1H (J = 6.9 Hz) C**H**(CH_3)Br). HRMS (+ESI): *m/z* calculated for $[M+Na]^+$: 273.9509. Found: 273.9493. Crystallographic quality crystals were grown by slow evaporation of an acetonitrile solution. *Crystal data* – $C_7H_8NO_4Br$, *M* 250.05, orthorhombic, space group *Pbca*, a = 10.4530(19), b = 8.8751(13), c = 19.529(4) Å, V = 1811.8(5) Å^3, Z = 8, $F(000)$ = 992, μ(Mo-Kα) = 0.091 cm^{-1}, T = 90(2) K, 2472 unique reflections. Refinement of 119 parameters converged at final R_1 = 0.034, wR_2(all data) = 0.078.

2.7. N-(α-Bromo-α-methylpropionyl)-chitosan **3a**

Chitosan (90% DD, 400.1 mg) was stirred in 0.3 M AcOH/0.2 M AcONa(aq) buffer (40.0 mL) at ambient temperature for 1 h to dissolve and a solution of **2a** (602.1 mg, 2.280 mmol) in $CHCl_3$ (15.0 mL) was added. The mixture was stirred vigorously for 3 h at ambient temperature and the suspension was poured into water. A 1.0 M NaOH(aq) solution was added to give a basic mixture (pH 13) which contained a precipitate. The suspension was centrifuged and the supernatant liquid discarded. The $CHCl_3$ layer under the solid was removed via pipette. The precipitate was resuspended in water then centrifuged until the supernatant liquid was neutral (3×). The solid was resuspended in EtOH and centrifuged (2×). The solid was resuspended in EtOH, reduced and dried under vacuum to give 278.7 mg of a cream solid. The impure solid was stirred in $CHCl_3$ and filtered. The solid was washed sequentially with $CHCl_3$, EtOH and petroleum ether and left to dry under vacuum for 3 h to give 187.8 mg of a cream solid **3a**. Calculated for $(Chitin)_{0.10}(Chitosan)_{0.82}$(*N*-(C(=O)C$(CH_3)_2$Br)-chitosan)$_{0.08}$·0.7$H_2O$: C, 41.2; H, 6.9; N, 7.4; Br, 3.4. Found: C, 41.0; H, 7.2; N, 7.5; Br, 3.2. IR ($\tilde{\nu}$, cm^{-1}): 3357 (OH str) 2867 (CH str) 1651 (NH bend) 1592 (Amide III) 1418, 1376, 1319, 1259, 1150 (pyranose) 1057 (pyranose) 1074 (pyranose) 1025 (pyranose) 895. ^{1}H NMR (δ, ppm, d_4-AcOD/D_2O): 2.22 (*w* s, initiator C**H**$_3$) 2.98 (*w* s, initiator ring **H**) 3.18 (s, chitosan **H**2) 3.71 and 3.90 (2× s, chitosan **H**3, **H**4, **H**5, **H**6). GPC (g mol^{-1}): M_p = 569,100: M_w = 1,568,800; M_n = 168,500; PDI = 9.3.

2.8. N-(α-Bromopropionyl)-chitosan **3b**

Chitosan (90% DD, 200.5 mg) was stirred in 0.3 M AcOH/0.2 M AcONa(aq) buffer (20.0 mL) at 60 °C for 90 min. EtOH (5.0 mL) was added to the initiator **2b** (261.6 mg, 1.05 mmol) then CH_2Cl_2 (5.0 mL) was added to completely dissolve the solid. The organic solution was added to the yellow chitosan solution and stirred at 60 °C for 3 h and poured into NaOH(aq) (10.0 mL) to form a white gel-like precipitate. The mixture was centrifuged in H_2O (2×) and EtOH (2×). The basic mixture was filtered and the solid washed with water until neutral, MeOH and diethyl ether. The cream solid was air-dried, further dried under vacuum for three nights and ground up to give 175.6 mg of a fibrous cream solid **3b**. Calculated for $(Chitin)_{0.10}(Chitosan)_{0.89}$(*N*-(C(=O)C$HCH_3$Br)-chitosan)$_{0.01}$·0.3$H_2O$: C, 43.5; H, 6.9; N, 8.1; Br, 0.5. Found: C, 43.5; H, 7.7; N, 8.1; Br, 0.3. IR ($\tilde{\nu}$, cm^{-1}): 3357 (OH str) 2867 (CH str) 1651 (NH bend) 1592 (Amide III) 1418, 1376, 1319, 1259, 1150 (pyranose) 1057 (pyranose) 1074 (pyranose) 1025 (pyranose) 895. ^{1}H NMR (δ, ppm, d_4-AcOD/D_2O): 3.17 (s, chitosan **H**2) 3.71 and 3.90 (2× s, chitosan **H**3, **H**4, **H**5, **H**6). GPC (g mol^{-1}): M_p = 239,000; M_w = 419,200; M_n = 138,200; PDI = 3.0.

2.9. "Grafting-from" N-(α-bromo-α-methylpropionyl)-chitosan-graft-poly(OEGMA) **4a**

The macroinitiator **3a** (8% DS, 49.7 mg) was stirred in 0.3 M AcOH/0.2 M AcONa(aq) solution (20.0 mL) at 60 °C for 1 h and cooled to ambient temperature. A solution of CuBr (6.4 mg, 45 μmol) in 1:1 EtOH/H_2O (2.0 mL) was added to 2,2′-bipyridine (bipy) (14.2 mg, 90.9 μmol) and the resulting brown solution was added to the chitosan solution. A solution of the monomer OEGMA (3.0 mL, 6.8 mmol) in 1:1 EtOH/H_2O (4.0 mL) was added immediately after the catalyst solution and the clear brown solution was stirred at ambient temperature for 24 h. The resulting pink reaction mixture was exposed to air and dialysed for four nights. The clear solution was reduced in volume to give 143.8 mg of a white film **4a**. IR ($\tilde{\nu}$, cm^{-1}): 3357 (OH str) 2872 (CH str) 1726 (C=O str) 1651 (NH bend) 1592 (amide III) 1350 (CH bend) 1150 (pyranose) 1100 (CO str) 1074 (pyranose) 1057 (pyranose) 1030 (CO str) 748 (CH rock). ^{1}H NMR spectroscopy gave an estimate of 93 wt% poly(-OEGMA) in the comb. GPC (g mol^{-1}): M_p = 54,800; M_w = 204,300; M_n = 51,700; PDI = 4.0.

2.10. α-(2,5-Oxo-1-pyrrolidyl)oxy-2-methylpropionate-ω-bromo-poly(OEGMA) **5a**

The reagents CuBr (33.0 mg, 0.23 mmol) and bipy (72.2 mg, 0.46 mmol) were dissolved in 1:1 EtOH/H_2O (5.0 mL) and this solution was added to the stirred monomer OEGMA (9.7 mL, 22 mmol). The initiator **2a** (114 mg, 0.46 mmol) was dissolved in 1:1 EtOH/H_2O (4.0 mL) and degassed before addition to the monomer solution. The bulk of the viscous brown solution was stirred overnight for 18 h to form a green/brown solution. The solution was dialysed and reduced in volume to give 7.2 g of a clear oil **5a**. IR ($\tilde{\nu}$, cm^{-1}): 2872 (CH str) 1726 (C=O str) 1452, 1350 (CH bend) 1246, 1100 (CO str) 1030 (CO str) 946, 853, 748 (CH rock). ^{1}H NMR (δ, ppm, D_2O): 0.98 and 1.13 (2× s, 3H, C**H**$_3$) 1.96 (s, 2H, C**H**$_2$) 3.44 (s, 3H, ROC**H**$_3$) 3.75 (m, 32H, ROC**H**$_2$C**H**$_2$OR′) 4.24 (m, 2H, RC(=O)OC**H**$_2$R′). GPC (g mol^{-1}): M_p = 13,600; M_w = 15,900; M_n = 12,600; PDI = 1.3. Target M_n (100% conversion) = 23,000 g mol^{-1}.

2.11. α-(2,5-Oxo-1-pyrrolidyl)oxypropionate-ω-bromo-poly(OEGMA) **5b**

The reagents CuBr (46.0 mg, 0.32 mmol) and bipy (100.0 mg, 0.64 mmol) were dissolved in 1:1 EtOH/H_2O (3.0 mL). The brown solution was added to a stirred solution of the monomer OEGMA (22.7 mL, 51.6 mmol) in 1:1 EtOH/H_2O (10.0 mL). A solution of **2b** (184.8 mg, 0.640 mmol) in the same solvent (10.0 mL) was added to the monomer solution. Aliquots of the reaction mixture were re-

moved and characterised. The bulk of the solution was dissolved in EtOH and H_2O then dialysed and reduced in volume to give 5.5 g of a viscous colourless oil **5b**. IR ($\tilde{\nu}$, cm^{-1}): 2872 (CH str) 1726 (C=O str) 1452, 1350 (CH bend) 1246, 1100 (CO str) 1030 (CO str) 946, 853, 748 (CH rock). ^1H NMR (δ, ppm, D_2O): 0.96 (s, 3H, C**H**$_3$) 1.93 (s, 2H, C**H**$_2$) 3.38 (s, 3H, ROC**H**$_3$) 3.65 (m, 32H, ROC**H**$_2$C**H**$_2$OR′) 4.18 (m, 2H, RC(=O)OC**H**$_2$R′). GPC (g mol^{-1}): M_p = 47,900; M_w = 64,200; M_n = 45,200; PDI = 1.4. Target M_n (100% conversion): 38,500 g mol^{-1}.

2.12. "Grafting-to" N-(α-bromo-α-methylpropionyl)-chitosan-graft-poly(OEGMA) **6a**

Chitosan (90% DD, 133.7 mg) was heated in 0.3 M AcOH/0.2 M AcONa(aq) buffer (10.0 mL) and a solution of **5a** (M_n = 8500 g mol^{-1}, 133.4 mg) in the same buffer (5.0 mL) was added to the chitosan solution. The viscous mixture was stirred at 70 °C for 5 h. The yellow solution which contained a small amount of precipitate was poured into basic EtOH and the cloudy white mixture was left to settle overnight. The suspension in EtOH was centrifuged and the supernatant liquid discarded (4×). The pale yellow solid was suspended in water and dialysed. The dialysed mixture was centrifuged and the resuspended solid was freeze-dried to give 68.0 mg of a white flaky solid **6a**. Calculated for $(Chitin)_{0.10}(Chitosan)_{0.90}\cdot 0.1poly(OEGMA)\cdot 1.6H_2O$: C, 41.7; H, 7.8; N, 5.8; Br, 0.0. Found: C, 41.3; H, 6.8; N, 7.4; Br, 0.0. IR ($\tilde{\nu}$, cm^{-1}): 3357 (OH str) 2872 (CH str) 1725 (C=O str) 1651 (chitosan NH bend) 1592 (chitosan amide III) 1350 (CH bend) 1150 (pyranose) 1074 (pyranose) 1057 (pyranose) 1025 (pyranose) 748 (CH rock). The ^1H NMR spectrum gave an estimate of 24 wt% poly(OEGMA) in the comb. GPC (g mol^{-1}): M_p = 76,700; M_w = 362,100; M_n = 47,200; PDI = 7.7.

2.13. "Grafting-to" N-(α-bromopropionyl)-chitosan-graft-poly(OEGMA) **6b**

Chitosan (90% DD, 201.2 mg) was stirred in 0.3 M AcOH/0.2 M AcONa(aq) buffer (20.0 mL) at 60 °C for 1 h. A solution of **5b** (M_n = 45,200 g mol^{-1}, 203.9 mg) in the same buffer (5.0 mL) was added and stirred at 60 °C for 4 h. A NaOH(aq) solution (10.0 mL) was added to give a basic white cloudy suspension (pH 12). The mixture was centrifuged and the supernatant discarded (3×) then the solid resuspended and dialysed. The dialysed mixture was reduced in volume and the solid was freeze-dried overnight to give 190.2 mg of a fluffy white solid **6b**. IR ($\tilde{\nu}$, cm^{-1}): 3357 (OH str) 2872 (CH str) 1725 (C=O str) 1651 (chitosan NH bend) 1592 (chitosan amide III) 1350 (CH bend) 1150 (pyranose) 1074 (pyranose) 1057 (pyranose) 1025 (pyranose) 748 (CH rock). ^1H NMR spectroscopy gave an estimate of 6 wt% poly(OEGMA) in the comb. GPC (g mol^{-1}): M_p = 600,900; M_w = 1,543,200; M_n = 159,300; PDI = 9.7.

3. Results and discussion

Chitosan-*graft*-poly(OEGMA) copolymers were prepared by two synthetic routes with the polymerisation of poly(OEGMA) proceeding by an ATRP reaction with a copper(I)/bipyridine catalyst. In the "grafting-from" method, chitosan was reacted with a succinimide-containing initiator to prepare a chitosan-based macroinitiator. OEGMA was polymerised in the presence of the resulting chitosan macroinitiator to give the comb polymer. The "grafting-to" method involved the ATRP of OEGMA in an aqueous solution to give poly(-OEGMA) with a succinimide end group. The prepared polymer was subsequently attached to chitosan in an acetic acid solution to form a grafted copolymer.

3.1. Initiators

The initiator **2a** was prepared from BIBB and NHS (Lecolley et al., 2004) and was found to slowly decompose with time. The initiator was kept under an argon atmosphere and the purity checked by ^1H NMR spectroscopy prior to use in subsequent reactions. When necessary, **2a** was dissolved in CH_2Cl_2 and the organic solution washed with dilute aqueous base, acid and base again then reduced in volume to get the purified compound. The second initiator **2b** was prepared by the reaction of NHS and BPA with DCC (Lecolley et al., 2004) and also slowly decomposed with time as determined by ^1H NMR spectroscopy. A CH_2Cl_2 solution of impure **2b** was typically rewashed with base and water then reduced to dryness to obtain the pure solid. The structures of **2a** and **2b** were confirmed by single-crystal X-ray crystallography.

The initiator with two methyl groups (**2a**) crystallised in the chiral orthorhombic space group $P2_12_12_1$ with two chemically identical but crystallographically distinct molecules in the asymmetric unit (Fig. 3a). Both molecules had flattened five-membered rings as a consequence of the presence of the imide group. The initiator with one methyl group (**2b**) crystallised in the orthorhombic space group *Pbca* with one complete molecule in the asymmetric unit (Fig. 3b). The five-membered ring was again flattened by the presence of the imide group. The bond lengths in **2b** were very similar to those for the related compound **2a**. Compounds **2a** and **2b** showed no unusual bond lengths in comparison to molecules

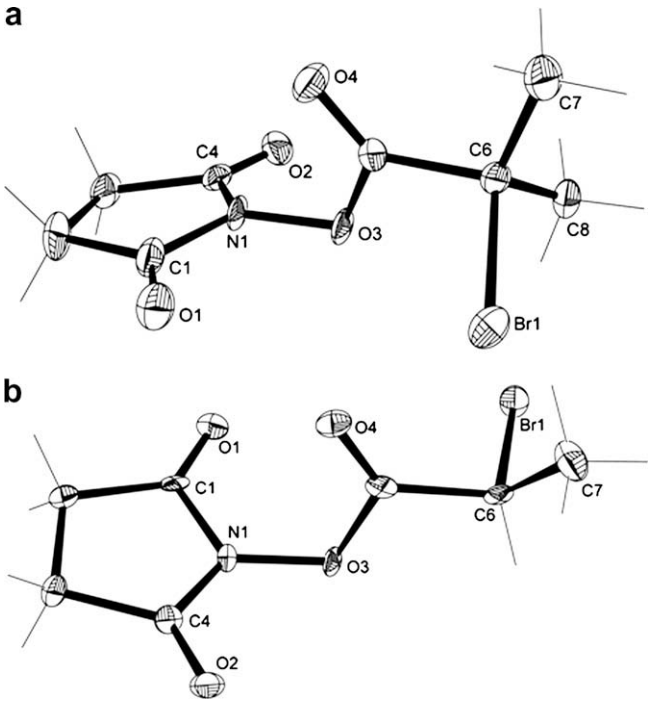

Fig. 3. (a) A perspective view (crystallographic numbering) of one of the two molecules of **2a** in the asymmetric unit with the thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å): C1–N1 1.383(5), C4–N1 1.394(4), O3–N1 1.389(3), C5–O3 1.387(5), C5–O4 1.191(4), C6–Br1 1.978(4). Selected bond angles (°): O1–C1–N1 124.2(3), C1–N1–C4 117.3(3), O2–C4–N1 125.1(3), C1–N1–O3 120.9(3), O3–N1–C4 120.6(3), C5–O3–N1 111.7(3), O3–C5–C6 110.4(3), O4–C5–O3 123.2(3). (b) A perspective view (crystallographic numbering) of the single molecule **2b** in the asymmetric unit with the thermal ellipsoids drawn at the 50% probability level. Selected bond lengths (Å): C1–N1 1.390(5), C4–N1 1.397(5), O3–N1 1.384(4), C5–O3 1.391(4), C5–O4 1.197(4), Br1–C6 1.978(3). Selected bond angles (°): O1–C1–N1 124.3(3), C1–N1–C4 116.1(3), O2–C4–N1 124.3(3), O3–N1–C1 121.0(3), O3–N1–C4 121.6(3), N1–O3–C5 111.5(3), O3–C5–C6 109.1(3), O4–C5–O3 122.9(3).

of a similar type (Cambridge Structural Database version 5.28) (Allen et al., 1991).

3.2. Chitosan macroinitiator

The "grafting-from" method involved modification of the polysaccharide by attaching initiating sites to the chitosan backbone and subsequently using this macroinitiator in the ATRP reactions. The succinimide group in the two respective compounds **2a** and **2b** was expected to be a good leaving group and readily undergo substitution with nucleophiles such as the amines present on the chitosan backbone (Ladmiral, Monaghan, Mantovani, & Haddleton, 2005; Lecolley et al., 2004). Therefore, the initiator **2a** or **2b** was reacted with chitosan in an interfacial reaction to form the polymer **3a** or **3b**, respectively, under a variety of reaction conditions (Table 1). The variables that were examined were the reaction ratio between chitosan and the initiator, the solvent system, length of reaction time and temperature.

The reaction ratio had a significant effect on the degree of substitution (DS) of the polymers **3a**. The products formed with reaction ratios of less than one equivalents of initiator (compared to the chitosan) showed no evidence of substitution under the reaction conditions examined. Substitution occurred for four of the six polymers of **3a** formed with reaction ratios higher than one equivalent of initiator to chitosan. The solvent system was an additional factor in the success of the reaction. The change from MeOH to $CHCl_3$ as the second solvent for a reaction at 40 °C for 5 h under basic conditions to give 0 and 1 mol % substitution, respectively, suggested that $CHCl_3$ was a better solvent for the initiator. The length of reaction time influenced the DS values of the products. An increase in the reaction time at ambient temperature in an acidic/$CHCl_3$ system with a 1:2 molar ratio of chitosan and initiator from 2 h to 3 h gave an increase in substitution from 2 to 8 mol %, respectively, for the products. Reactions at ambient temperature gave products with significant amounts of bromine (and therefore higher DS values) compared to reactions at 40 °C or 60 °C. The DS values of **3b** were very low under all of the reaction conditions investigated.

The DS values of the chitosan macroinitiators **3a** and **3b** were estimated from the microanalytical data and a selection of the data are shown in Table 2. The amount of bromine in **3a** varied from 0.0 to 4.6 wt% which showed the range of substitution of the products with different reaction conditions. The low amounts of bromine in the analyses for **3b** were within the error of this technique (±0.4%) and indicated that the reaction of **2b** and chitosan **1** to form a chitosan macroinitiator **3b** was unsuccessful under all the reaction conditions investigated. The IR spectra of the polymers with high DS values showed small peaks assigned to the initiating groups but these results were not quantifiable and were less informative than the elemental analyses.

The low DS values for **3b** (and to a lesser extent **3a**) prepared under various reaction conditions may have arisen from the reactive nature of the initiators **2a** and **2b**. It was proposed that the initiators underwent hydrolysis in the reaction conditions investigated and this reaction was preferred to the desired nucleophilic substitution of the chitosan amines at the carbonyl centre of the initiator. The initiator **2a** was considered more successful than the initiator **2b** for the formation of the chitosan macroinitiator as this compound was more stable under the reaction conditions and underwent the desired reaction. The degradation of **2a** was minimised by low temperatures, well-chosen solvents and high reaction ratios.

3.3. "Grafting-from" method

The chitosan macroinitiator **3a** was used in the subsequent "grafting-from" ATRP reaction of OEGMA in an acidic solution to form the copolymer **4a**. The products varied in appearance and in the amount of synthetic polymer that was present (Table 3). The white sticky solid had the lowest amount of associated synthetic polymer at 55 wt%. The two films contained much more synthetic polymer in the samples. The IR spectra showed peaks assigned to both poly(OEGMA) and chitosan although the intensities were not quantified as the ^{1}H NMR spectra were more informative.

The MW values of the three polymers were determined by GPC (Fig. 4). All three samples showed the presence of two peaks. The

Table 1
The reaction conditions used for the reaction of **2** and chitosan **1** to form the macroinitiator **3**.

Initiator	Ratio[a]	Solvent 1[b]	Solvent 2[c]	Time (h)	Temperature (°C)	DS (%)
2a	1:0.5	Buffer	$CHCl_3$	4.5	60	0
2a	1:0.9	Buffer	$CHCl_3$	2	Ambient	0
2a	1:0.9	Base	$CHCl_3$	2	Ambient	0
2a	1:1.1	Buffer	$CHCl_3$	2	Ambient	2
2a	1:1.1	Buffer	$CHCl_3$	3	Ambient	8
2a	1:2	Base	$CHCl_3$	2	Ambient	11
2a	1:2	Base	$CHCl_3$	5	40	1
2a	1:2	Base	MeOH	5	40	0
2a	1:2	Buffer	MeOH	5	40	0
2b	1:0.45	Buffer	CH_2Cl_2/EtOH	3	60	1
2b	1:0.45	Base	CH_2Cl_2/EtOH	3	60	1
2b	1:0.9	Buffer	CH_2Cl_2/EtOH	3	60	1
2b	1:0.9	Base	CH_2Cl_2/EtOH	3	60	1
2b	1:0.5	Buffer	$CHCl_3$	4.5	60	0
2b	1:2	Buffer	$CHCl_3$	2	50	0
2b	1:2	Buffer	$CHCl_3$	4	50	1
2b	1:2	Buffer	$CHCl_3$	4	50	0
2b	1:2	Buffer	$CHCl_3$	4	50	0
2b	1:2	Base	$CHCl_3$	5	40	0
2b	1:2	Buffer	MeOH	5	40	0
2b	1:2	Base	MeOH	5	40	0

[a] The reaction ratio (mol:mol) between chitosan and the initiator (2,5-oxo-1-pyrrolidyl)oxy-2-bromo-2-methylpropionate.
[b] The solvent chitosan was dissolved or dispersed in. The buffer was 0.3 M AcOH/0.2 M AcONa(aq) solution and the base was 0.1 M NaOH(aq) solution.
[c] The solvent that the initiator was dissolved in.

Table 2
The DS values and microanalytical data for a selection of the polymers **3a** and **3b**.

Product	DS (%)	Formula[a]	Calculated (%) *Found* (%)			
			C	H	N	Br
3a	0	$(CH)_{0.10}(CS)_{0.90}\cdot 0.4H_2O$	43.1	7.0	8.1	0.0
			43.5	*7.3*	*7.6*	*0.1*
3a	1	$(CH)_{0.10}(CS)_{0.89}(N\text{-}(COCMe_2Br)\text{-}CS)_{0.01}\cdot 0.1H_2O$	44.4	6.8	8.3	0.5
			44.4	*7.6*	*7.9*	*0.5*
3a	2	$(CH)_{0.10}(CS)_{0.88}(N\text{-}(COCMe_2Br)\text{-}CS)_{0.02}\cdot 0.8H_2O$	41.3	7.1	7.7	0.9
			41.7	*6.9*	*7.3*	*0.8*
3a	8	$(CH)_{0.10}(CS)_{0.82}(N\text{-}(COCMe_2Br)\text{-}CS)_{0.08}\cdot 0.7H_2O$	41.2	6.9	7.4	3.4
			41.0	*7.2*	*7.5*	*3.2*
3a	11	$(CH)_{0.10}(CS)_{0.79}(N\text{-}(COCMe_2Br)\text{-}CS)_{0.11}\cdot 1.0H_2O$	39.9	6.9	7.0	4.4
			39.9	*7.0*	*7.0*	*4.6*
3b	0	$(CH)_{0.10}(CS)_{0.90}\cdot 0.8H_2O$	41.4	7.2	7.8	0.0
			41.5	*7.0*	*7.9*	*0.0*
3b	1	$(CH)_{0.10}(CS)_{0.89}(N\text{-}(COCHMeBr)\text{-}CS)_{0.01}\cdot 0.7H_2O$	41.7	7.1	7.8	0.4
			41.9	*7.7*	*7.0*	*0.3*

[a] The formulae in the table refer to the following sugar units: CH, chitin; CS, chitosan; *N*-(COCMe$_2$Br)-CS, *N*-(2-bromo-2-methylpropionamide)-chitosan; *N*-(COCHMeBr)-CS, *N*-(2-bromopropionamide)-chitosan.

Table 3
The effect of the reaction ratio on the appearance and amount of synthetic polymer for the comb polymer **4a** and the MW values of the products determined by GPC in acetate buffer.

Macroinitiator **3a**		Product **4a**			GPC results				
% sub	Mass (mg)	Yield (mg)	wt% Polymer[a]	Appearance	No.of Peaks	M_p	M_w	M_n	PDI
2	12.0	20.2	55	Sticky white solid	Two	308,900	899,600	267,500	3.4
						6300	7400	5900	1.2
8	49.7	143.8	93	White film	Two	54,800	204,300	51,700	4.0
						Overlapped with solvent, undetermined			
11	11.3	74.8	92	Clear film	Two	36,500	56,300	27,900	2.0
						1700	1700	1500	1.1

[a] Determined by ^{1}H NMR spectroscopy.

peak with a longer retention time was attributed to poly(OEGMA) oligimers which were unable to be removed by dialysis or washing of the samples. These impurities meant that the amount of grafted poly(OEGMA) was unable to be reliably quantified. The large peak at a shorter retention time was assigned to the grafted chitosan polymer. The retention times of the grafted polymer peaks increased as the amount of initiating sites increased in the macroinitiators. This observation was attributed to increasing amounts of grafted poly(OEGMA) lowering the hydrodynamic volume of the copolymers by affecting the conformation or by preventing aggregation. Gorochovceva and Makuška (2004) also reported that the reduced viscosity of chitosan-*graft*-PEG was smaller than the viscosity of the parent polymer chitosan, in spite of the increased MW of the copolymer. This result was attributed to the PEG groups preventing the approach of other molecules to the chitosan backbone and therefore minimising the amount of aggregation and hydrogen-bonding in solution, which is well-known for chitosan (Anthonsen, Vårum, Hermansson, Smidsrød, & Brant, 1994; Hu et al., 2007; Nilza de Carvalho Canella & Balaban Garcia, 2001). However, this would need to be confirmed by further studies.

One of the major difficulties in preparing graft copolymers is the formation of homopolymer which can be difficult to remove (Jenkins & Hudson, 2001). The presence of the unreacted monomer in our "grafting-from" polymers meant that the "grafting-to" synthetic route was investigated to prepare chitosan-*graft*-poly(OEGMA) copolymers. An advantage of this synthetic method is the defined nature of the side chains and therefore of the resulting polymers (Gorochovceva & Makuška, 2004; Huang et al., 2005; Kurita et al., 1999; Lebouc et al., 2005). The MW can be selected and experimentally determined before attachment to the polymer backbone.

3.4. ATRP of poly(OEGMA)

Telechelic poly(OEGMA) was successfully prepared by ATRP in an aqueous ethanolic solution with a copper(I)/bipyridine catalyst and the initiators **2a** or **2b** to give the polymers **5a** and **5b**, respectively. These ambient temperature polymerisations were monitored by ^{1}H NMR spectroscopy and the purified aliquots analysed by GPC. The MW values were in reasonable agreement with the target MW values and the PDI values were typically 1.3 and 1.4. The linear relationships between the number-average molecular weights and conversions for the two polymers, respectively, were consistent with an ATRP mechanism (Fig. 5). The two polymers each contained a pendant succinimide group that was suitable to prepare chitosan comb polymers by the "grafting-to" method.

3.5. "Grafting-to" method

The reaction of chitosan with poly(OEGMA) **5a** was investigated under a number of reaction conditions. The effects of the MW of the chitosan, reaction ratio between the chitosan and poly(OEGMA), reaction time and temperature on the resulting grafted copolymers **6a** and **6b** were examined (Table 4). The extent of grafting and MW values of the combs were evaluated by ^{1}H NMR spectroscopy and GPC, respectively.

The effect of the MW of the chitosan on the copolymer **6a** was examined with two chitosan samples of differing MW values as the differences in viscosity of the polymers and the resulting reaction solutions may have influenced the ease of reactivity and thus the grafting efficiency. Both products had trace amounts of the synthetic polymer incorporated although the appearance of the polymers varied from each another. The reaction ratio between the

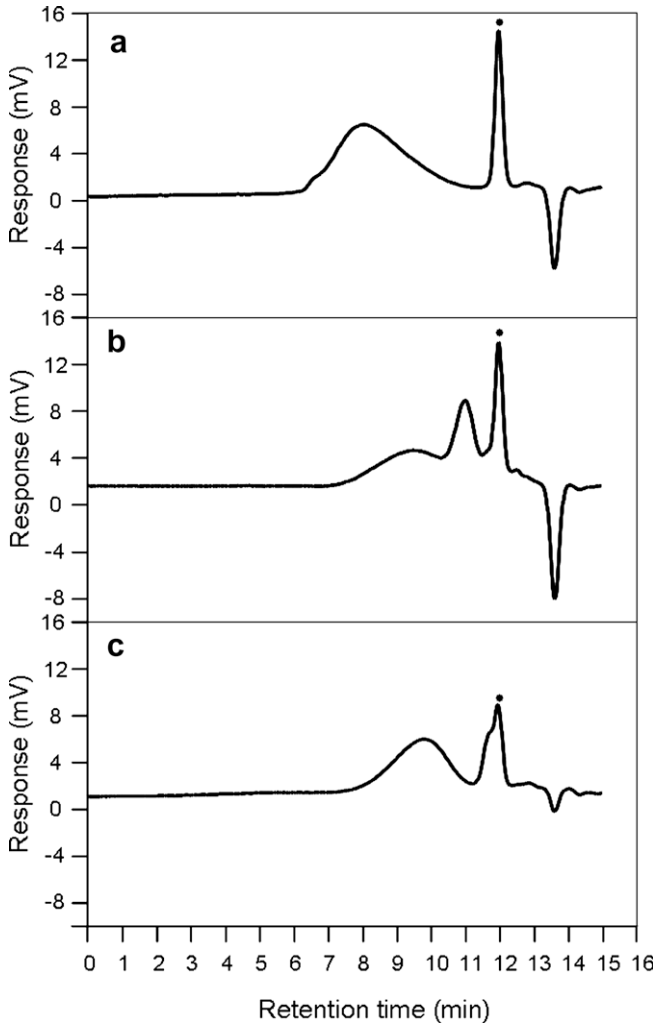

Fig. 4. The GPC traces of (a) chitosan, and the "grafting-from" product **4a** prepared with (b) a 2% macroinitiator or (c) a 8% macroinitiator. The star indicates the solvent peak.

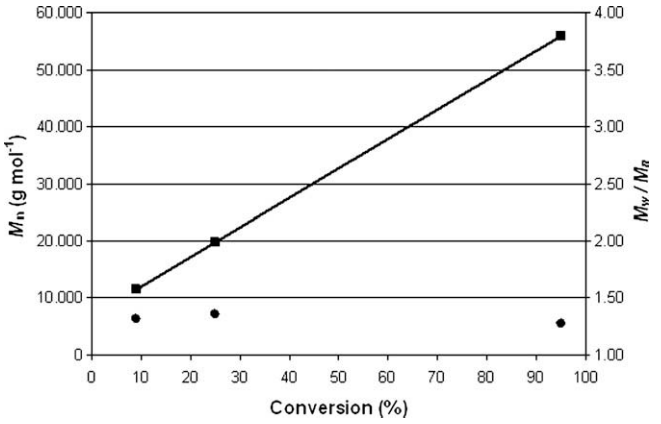

Fig. 5. A representative plot of the number-average molecular weight M_n, and PDI values, for the polymer **5b** as the conversion increased.

chitosan and poly(OEGMA) was also investigated by varying the ratio from 1:1 to 1:3. The product **6a** when formed with equal amounts of the polymers contained trace amounts of poly(OEGMA), in comparison to the polymer formed at a higher ratio of 1:2 which contained 8 wt% of the synthetic polymer. A further increase in the reaction ratio to 1:3 (although with a shorter reaction time) gave a product with only trace amounts of poly(OEGMA) detectable by ^{1}H NMR spectroscopy. The product prepared at 70 °C for 5 h with a one to one ratio of chitosan and synthetic polymer contained the most synthetic polymer in the comb (24 wt%) of the prepared samples.

The GPC retention times of the products **6a** were longer than for the parent chitosan and shorter than for poly(OEGMA). These results were once again consistent with significant changes in the hydrodynamic volumes due to the incorporation of poly(OEGMA) in the grafted copolymers. This was attributed to the effect of poly(OEGMA) on the conformation and hydrogen-bonding of the chitosan backbone. The presence of a single peak in the GPC chromatograph indicated that the synthetic polymer was successfully removed during purification and that the product was indeed a grafted copolymer (Fig. 6). This observation was supported by the presence of peaks assigned to both chitosan and poly(OEGMA) in the ^{1}H NMR spectra.

Three chitosan polymers with differing MW values were reacted with poly(OEGMA) at 60 °C for three and a half hours to form the products **6b**. However, there was no evidence of any grafting under the reaction conditions attempted for any of these samples. These samples were prepared with a equal amount of chitosan and poly(OEGMA) so the reaction ratio was subsequently examined. The product **6b** prepared with the largest ratio of synthetic polymer gave a small but measurable amount of 6 wt% grafting of poly(OEGMA) as determined by ^{1}H NMR spectroscopy. The MW values as determined by GPC for the sample which contained 6 wt% of synthetic polymer were also lower than for the parent chitosan and larger than for poly(OEGMA). The changes in the MW values were indicative of a change in the hydrodynamic volume for the product compared to the two starting polymers. The presence of a single peak in the GPC trace indicated that the comb was successfully purified with no starting polymers present.

The effect of reaction ratio and length of reaction time was investigated at 70 °C (Table 5). Although there was no evidence

Table 4
The effect of various reaction conditions on the formation of the copolymer **6a** (The synthetic polymer used was **5a** with M_n = 12,600 g mol^{-1}).

CS M_n(10^3)	Conditions			Polymer		GPC results			
	Ratio[a]	Temperature (°C)	Time (h)	(wt%)	Appearance	M_p	M_w	M_n	PDI
40	1:1	60	16	Trace	Cream powdery coating	Below detection limit			
384	1:1	60	16	Trace	Cream film	1,095,500	2,113,700	216,300	9.8
384	1:2	60	16	8	Cream film	1,207,300	2,633,000	235,300	11.2
384	1:3	60	4	Trace	Cream solid	259,900	373,500	75,300	5.0
384	1:1	70	5	24	White flaky solid	76,700	362,100	47,200	7.7

The reaction solvent was 0.3 M AcOH/0.2 M AcONa(aq) solution.
[a] The reaction ratio (w/w) between chitosan and poly(OEGMA).

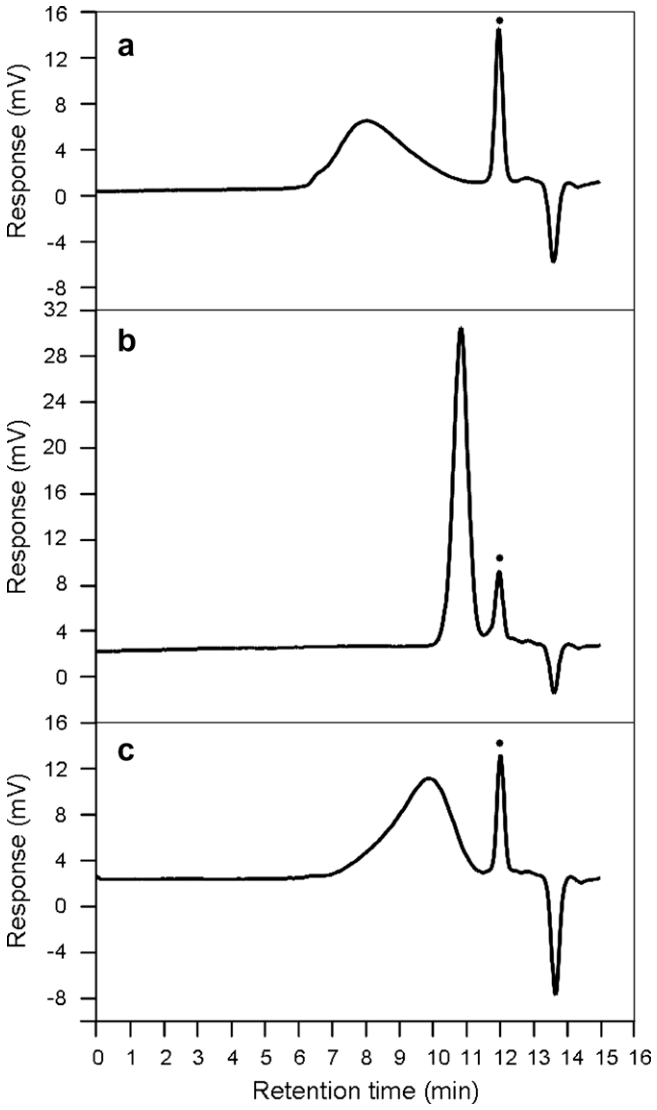

Fig. 6. The GPC traces of (a) chitosan, (b) poly(OEGMA) **5a** and (c) the grafted copolymer **6a**. The star indicates the solvent peak. The presence of a single peak in (c) indicated that the product was a grafted polymer instead of a mixture of chitosan and poly(OEGMA).

of grafting for any of the six products prepared at this temperature the products varied in appearance for the two different reaction times. The polymers prepared with a long reaction time of 23 h formed films which gave a frosted appearance to the round bottom flask. These films were unable to be scraped off the glass but were dissolved in an acetic acid buffer for analysis. Therefore the significant difference in appearance compared to the initial chitosan polymer (which was a cream solid) was suggestive of slight modification of the chitosan polymers even though the amount of synthetic polymer was below the detection limit for ^{1}H NMR spectroscopy. The higher reaction temperature may have led to faster hydrolysis of the *N*-hydroxysuccinimide group and an unreactive synthetic polymer, leading to no detectable poly(OEGMA) in the resulting products.

The polymers prepared with the initiator **2a** were more reactive to chitosan and gave "grafted-to" comb polymers with more synthetic polymer attached than the polymers prepared with **2b**. The polymers prepared with the initiator **2b** were less suitable for the reaction with chitosan and only one of the resulting products had a detectable amount of synthetic polymer incorporated. The presence of a single peak in the GPC traces of the grafted polymers prepared by the "grafting-to" synthetic route was consistent with the successful purification of these materials.

4. Conclusion

Chitosan-*graft*-poly(OEGMA) copolymers were successfully prepared by the ATRP of OEGMA by two synthetic routes. In the "grafting-from" method, chitosan was reacted with a succinimide-containing initiator to form a macroinitiator. OEGMA was successfully polymerised in the presence of the resulting chitosan macroinitiator in an acidic aqueous solution. The "grafting-from" copolymers were successfully prepared but were unable to be completely purified and contained unbound oligimers even after extensive washing. In the "grafting-to" method, poly(OEGMA) with a succinimide end group was prepared and subsequently attached to chitosan to form a copolymer. The "grafted-to" comb polymers were successfully prepared and were found to display a large change in physical appearance even with a low amount of grafted polymer incorporated in the copolymer. Two initiators were used and the initiator that contained two methyl groups was more successful in both synthetic routes. The initiator with one methyl group was less stable and decomposed more readily under the reaction conditions used. The hydrodynamic volumes of the copolymers were lower than for chitosan and this was attributed to a significant effect of the poly(OEGMA) on the conformation and hydrogen-bonding of the chitosan polymers.

Table 5
The effect of the reaction conditions on the amount of grafting of the copolymer chitosan-*graft*-poly(OEGMA) **6b** (The synthetic polymer used was **5b** with M_n = 45,200 g mol^{-1}).

Chitosan M_n(10^3 g mol^{-1})	Ratio[a]	Temperature(°C)	Time(h)	Polymer(wt%)	Appearance
7	1:1	60	3.5	0	Pale yellow powdery solid
40	1:1	60	3.5	0	Yellow powdery solid
384	1:1	60	3.5	0	Cream solid
384	1:1	60	4	0	Cream filmy powder
384	1:2	60	4	0	Cream filmy powder
384	1:4	60	4	6	Fluffy white solid
384	1:2	70	4	0	White powder
384	1:4	70	4	0	White filmy solid
384	1:6	70	4	0	White flaky solid
384	1:2	70	23	0	White film[b]
384	1:4	70	23	0	White film[b]
384	1:6	70	23	0	Cream film[b]

The reactions were carried out in 0.3 M AcOH/0.2 M AcONa(aq) solution.
[a] Chitosan to poly(OEGMA) reaction ratio (w/w).
[b] The film gave a frosted appearance to the round bottom flask and required dissolution in acetate buffer for further analysis.

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References

Allen, F. H., Davies, J. E., Galloy, J. J., Johnson, O., Kennard, O., Macrae, C. F., et al. (1991). The development of versions 3 and 4 of the Cambridge Structural Database System. *Journal of Chemical Information and Computer Sciences, 31*, 187–204.

Altomare, A., Burla, M. C., Camalli, M., Cascarano, G. L., Giacovazzo, C., Guagliardi, A., et al. (1999). SIR97: A new tool for crystal structure determination and refinement. *Journal of Applied Crystallography, 32*, 115–119.

Anthonsen, M. W., Vårum, K. M., Hermansson, A. M., Smidsrød, O., & Brant, D. A. (1994). Aggregates in acidic solutions of chitosans detected by static laser light scattering. *Carbohydrate Polymers, 25*, 13–23.

El Tahlawy, K., & Hudson, S. M. (2003). Synthesis of a well-defined chitosan graft poly(methoxy polyethyleneglycol methacrylate) by atom transfer radical polymerization. *Journal of Applied Polymer Science, 89*, 901–912.

Farrugia, L. J. (1999). WinGX suite for small-molecule single-crystal crystallography. *Journal of Applied Crystallography, 32*, 837–838.

Gorochovceva, N., & Makuška, R. (2004). Synthesis and study of water-soluble chitosan-*O*-poly(ethylene glycol) graft copolymers. *European Polymer Journal, 40*, 685–691.

Harris, D. C. (2003). *Quantitative chemical analysis*. New York: W.H. Freeman & Co.

Hu, Y., Du, Y., Yang, J., Tang, Y., Li, J., & Wang, X. (2007). Self-aggregation and antibacterial activity of *N*-acylated chitosan. *Polymer, 48*, 3098–3106.

Huang, M., Shen, W., & Fang, Y. e. (2005). Synthesis of a novel chitosan derivative having poly(ethylene oxide) side chains in aqueous reaction media. *Reactive & Functional Polymers, 65*, 301–308.

Jenkins, D. W., & Hudson, S. M. (2001). Review of vinyl graft copolymerization featuring recent advances towards controlled radical-based reactions: Illustrated with chitin/chitosan trunk polymers. *Chemical Reviews, 101*, 3245–3273.

Kulkarni, A. R., Lin, Y.-H., Liang, H.-F., Chang, W.-C., Hsiao, W. W.-W., & Sung, H.-W. (2006). A novel method for the preparation of nanoaggregates of methoxy polyethyleneglycol linked chitosan. *Journal of Nanoscience and Nanotechnology, 6*, 2867–2873.

Kurita, K. (2001). Controlled functionalization of the polysaccharide chitin. *Progress in Polymer Science, 26*, 1921–1971.

Kurita, K., Amemiya, J., Mori, T., & Nishiyama, Y. (1999). Comb-shaped chitosan derivatives having oligo(ethylene glycol) side chains. *Polymer Bulletin, 42*, 387–393.

Ladmiral, V., Monaghan, L., Mantovani, G., & Haddleton, D. M. (2005). A-Functional glycopolymers: New materials for (poly)peptide conjugation. *Polymer, 46*, 8536–8545.

Lebouc, F., Dez, I., Desbrières, J., Picton, L., & Madec, P.-J. (2005). Different ways for grafting ester derivatives of poly(ethylene glycol) onto chitosan: Related characteristics and potential properties. *Polymer, 46*, 639–651.

Lecolley, F., Tao, L., Mantovani, G., Durkin, I., Lautru, S., & Haddleton, D. M. (2004). A new approach to bioconjugates for proteins and peptides ("pegylation") utilising living radical polymerization. *Chemical Communications (Cambridge, United Kingdom)*, 2026–2027.

Liu, L., Li, F., Fang, Y. e., & Guo, S. (2006). Regioselective grafting of poly(ethylene glycol) onto chitosan and the properties of the resulting copolymers. *Macromolecular Bioscience, 6*, 855–861.

Munro, N. H., Hanton, L. R., Moratti, S. C., & Robinson, B. H. (2007). Synthesis and characterisation of chitosan combs by ATRP. *Advances in Chitin Science, 10*, 91–95.

Munro, N. H., Hanton, L. R., Robinson, B. H., & Simpson, J. (2008). Synthesis and characterisation of fluorescent chitosan derivatives containing substituted naphthalimides. *Reactive & Functional Polymers, 68*, 671–678.

Nilza de Carvalho Canella, K. M., & Balaban Garcia, R. (2001). Characterization of chitosan by gel permeation chromatography – influence of preparation method and solvent. *Quimica Nova, 24*, 13–17.

Otwinowski, Z., & Minor, W. (1997). Processing of X-ray diffraction data collected in oscillation mode. *Methods in Enzymology, 276*, 307–326.

Sheldrick, G. M. (1996). *SADABS – program for absorption correction*.

SAINT V4. (1996). Area detector control and integration software. SAINT.

Sheldrick, G. M. (2008). A short history of SHELX. *Acta Crystallographica, A64*, 112–122.

Wedmore, I., McManus, J. G., Pusateri, A. E., & Holcomb John, B. (2006). A special report on the chitosan-based hemostatic dressing: Experience in current combat operations. *The Journal of Trauma, 60*, 655–658.

Werle, M., & Bernkop-Schnürch, A. (2008). Thiolated chitosans: Useful excipients for oral drug delivery. *Journal of Pharmacy and Pharmacology, 60*, 273–281.

Zohuriaan-Mehr, M. J. (2005). Advances in chitin and chitosan modification through graft copolymerization: A comprehensive review. *Iranian Polymer Journal, 14*, 235–265.