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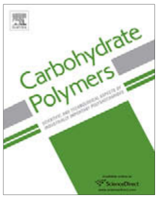

Preparation and graft copolymerisation of thiolated β-chitin and chitosan derivatives

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ABSTRACT

β-Chitin was extracted from squid pens and deacetylated to β-chitosan. Both polymers were treated with tosyl chloride, potassium thioacetate and sodium methoxide to form 6-mercaptochitin and 6-mercaptochitosan, respectively. The degrees of substitution were lower for the chitosan derivatives and both types of polymer were less substituted than related polymers prepared from α-chitin. The thiolated polymers were reacted with MMA to form grafted copolymers. The solvent had an influence on the success of the polymerisation with the chitosan polymers giving highly grafted materials in aqueous acetic acid solution.

1. Introduction

Chitin is the second most abundant biopolymer after cellulose and is typically extracted from crab and shrimp shells. The β-(1 → 4) polysaccharide is composed of predominately 2-acetamido-2-deoxy-D-glucopyranose sugars with minor amounts of the deacetylated sugar 2-amino-2-deoxy-D-glucopyranose (Muzzarelli, 1977). Deacetylation in strong base gives the related polymer chitosan which contains mainly amine groups at the C2 position. The properties of chitin and chitosan are affected by the polymorphic form, molecular weight (*MW*) and degree of deacetylation (*DD*).

The three polymorphic forms of chitin are related to the biological function of this structural polymer. α-Chitin is the most abundant form and is produced from crab and shrimp shells which are waste products from the fishing industry. β-Chitin, typically extracted from squid pens, is less widely used although it can have higher reactivity due to the weaker intermolecular hydrogen bonding between the chitin chains that occurs in β-chitin (Roberts, 1992). γ-Chitin is the least common form and has properties which are intermediate between those of α- and β-chitin (Rudall, 1963).

Thiolated chitosan polymers have been investigated for their metal binding capacity and selectivity (Cárdenas, Orlando, & Edelio, 2001; Lasko, Pesic, & Oliver, 1993), as macroinitiators for graft copolymerisation (Kurita, Hashimoto, Ishii, & Mori, 1996; Kurita, Hashimoto, Yoshino, Ishii, & Nishimura, 1996; Kurita, Inoue, & Harata, 2002) and for drug delivery and wound healing (Bernkop-Schnürch, Hornof, & Guggi, 2004; Guggi, Langoth, Hoffer, Wirth, & Bernkop-Schnürch, 2004; Hornof, Kast, & Bernkop-Schnürch, 2003; Hoyer, Schlocker, Krum, & Bernkop-Schnürch, 2008; Krauland, Guggi, & Bernkop-Schnürch, 2006; Masuko et al., 2005; Matsuda, Kobayashi, Itoh, Kataoka, & Tanaka, 2005; Werle & Bernkop-Schnürch, 2008). The thiol functional groups (R-SH) in the polymer can enhance the mucoadhesive properties of chitosan by forming disulfide bonds (R—S—S—R′) with cysteine residues in proteins (Bernkop-Schnürch et al., 2004). These materials are therefore suitable for drug delivery in a variety of physical forms such as gels, powders and films. The thiol groups can also dissociate to give hydrogen and sulfur free radicals which can initiate polymerisation with vinyl monomers like styrene and methyl methacrylate (MMA) (Kurita, Hashimoto, Ishii et al., 1996; Kurita, Hashimoto, Yoshino et al., 1996; Kurita et al., 2002).

Graft copolymers are an emerging group of polymers which consist of two types of polymers in one molecule. These materials can have properties which are different to both of the parent polymers (Jenkins & Hudson, 2001). 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 chitin derivative which has received attention for graft copolymerisation is 6-mercaptochitin. This derivative contains a thiol group in the 6-position which can easily react with vinyl monomers like styrene and MMA to form comb-like copolymers (Kurita, Hashimoto, Ishii et al., 1996; Kurita, Hashimoto, Yoshino et al., 1996; Kurita et al., 2002). Reaction at the 6-position leaves the amine free for further modifications. It is also possible for the polymers chains to form disulfide bonds which give rise to a cross-linked polymer (Hornof et al., 2003).

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Herein, we report the synthesis and characterisation of thiolated β-chitin and chitosan derivatives (Fig. 1) following the method reported by Kurita for α-chitin (Kurita et al., 1992). We show that this method is suitable for preparing thiolated derivatives of chitosan, although the degree of substitution (*DS*) was lower than for the products obtained from β-chitin. Chitin and chitosan were sequentially reacted with tosyl chloride, potassium thioacetate and sodium methoxide to form 6-mercaptochitin and 6-mercaptochitosan, respectively. These derivatives were characterised by microanalysis, IR and solid-state ^{13}C NMR spectroscopy. The products 6-mercaptochitin and 6-mercaptochitosan were used as the trunk polymers to form grafted copolymers with MMA. The obtained grafted copolymers were characterised by microanalysis and IR spectroscopy.

2. Experimental

2.1. Materials

Chitin was extracted from squid pens which were kindly donated by Otakou Fisheries, Dunedin, New Zealand. Tosyl chloride (TsCl) was purified before use by filtration of a benzene solution of the impure solid. The filtrate was reduced to dryness to give the pure reagent. MMA was distilled before use to remove inhibitor. Dimethylsulfoxide (DMSO) was distilled over CaH_2, stored over 4 Å molecular sieves and degassed prior to use. Pyridine was stored over KOH. All other reagents were purchased from Aldrich and used as received unless otherwise stated.

2.2. Characterisation

Microanalyses were used to estimate the *DS* values of the polymers and the amount of grafted MMA units per glycosidic unit in the copolymers. The molecular formulae of the various sugar units and the synthetic polymer poly(MMA) were entered into a Microsoft Excel spreadsheet to give the mass percentages. The ratios were varied to give an estimate of the percentages of each measured element C, H, N and S. These calculated values were compared to the experimental values obtained. The ratio of the various sugars or the degree of grafting of the copolymer was adjusted to give the closest fit to the experimental data for each sample with the lowest possible sum of squares.

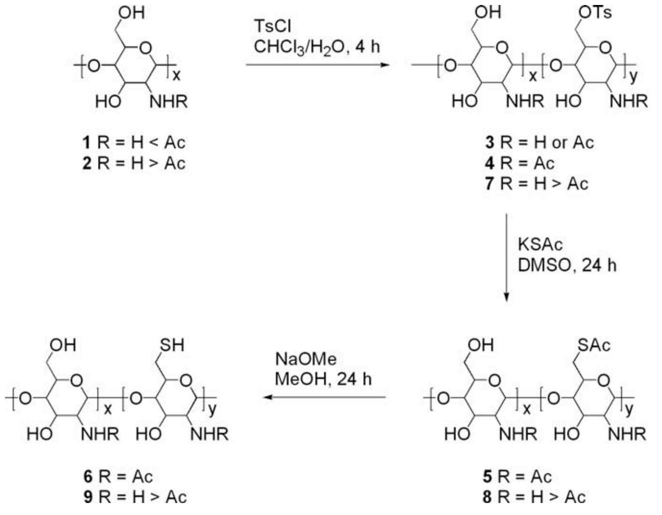

Fig. 1. The general reaction scheme showing the reactions involved to convert chitin **1** and chitosan **2** to the corresponding derivatives 6-mercaptochitin **6** and 6-mercaptochitosan **9**. Treatment of 6-tosylchitin **3** with acetic anhydride gave fully *N*-acetylated 6-tosylchitin **4**.

The ^{1}H NMR spectra were obtained on a Varian Unity Inova 500 (125) MHz spectrometer in d_4-AcOD/D_2O at 25 °C referenced to trimethylsilyl-2,2,3,3-tetradeuteropropionic acid (d_4-TSP). Solid-state ^{13}C NMR spectra were obtained at the University of Cambridge on a Bruker Avance 400 (100.5) MHz spectrometer with a 4 mm double resonance MAS probe. Spectra were acquired with CP/MAS techniques with TOSS. A sweep width of 40 kHz was used with a recycle delay of 3 s for the ^{13}C atoms. The conditions used for the samples were either a contact time of 1.5 ms with a spinning rate of 5 kHz and 20,000 scans or a contact time of 1.0 ms with a spinning rate of 10 kHz and 4000 scans. The peak assignments for the solid-state NMR data were made by comparison with the literature (Jang, Kong, Jeound, Lee, & Nah, 2004; Silverstein & Webster, 1998).

2.3. Extraction of *β*-chitin *1*

Dried cut-up squid pens (180.21 g) were stirred in 1.0 M NaOH(aq) solution (3.5 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 50.42 g of a white fluffy powder **1**. Calculated for $(\text{chitin})_{0.88}(\text{chitosan})_{0.12}\cdot 1.0H_2O$: 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. Chitosan *2*

The Kurita method was used to prepare β-chitosan (Tolaimate et al., 2000). β-Chitin **1** (12% *DD*, 25.01 g, 0.116 mol) was stirred in 10 M NaOH(aq) solution (1.0 L) at 80 °C for 2 h. The product was washed with water until neutral and dried overnight at 50 °C to give 15.81 g of a cream solid. The treatment was repeated to give 13.45 g of a cream solid **2**. Calculated for $(\text{chitin})_{0.20}(\text{chitosan})_{0.80}\cdot 0.8H_2O$: 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).

2.5. 6-Tosylchitin *3*

β-Chitin **1** (12% *DD*, 2.41 g, 13.1 mmol) was mechanically stirred at 100 rpm at ambient temperature in 10 M NaOH (50.0 mL) under reduced pressure for 3 h to swell. Crushed ice (12.5 g) was added and the mixture cooled in an ice bath. A solution of TsCl (27.99 g, 146.8 mmol) in $CHCl_3$ (100 mL) was added with vigorous stirring. The mixture was stirred for 2 h in ice and another 2 h at ambient temperature. The heterogeneous mixture was poured into an excess of distilled water and the supernatant liquid was decanted off the solid. The solid was repeatedly washed with distilled water and the supernatant liquid discarded until neutral. The mixture was filtered and the solid was washed with MeOH, diethyl ether then dried under vacuum to give 2.63 g of a fibrous cream solid **3**.

Calculated for $(\text{chitosan})_{0.18}(\text{6-tosylchitin})_{0.82}\cdot 1.2H_2O$: C, 46.8; H, 5.9; N, 4.1; S, 7.7. Found: C, 46.8; H, 5.8; N, 4.0; S, 7.8. IR ($\tilde{\nu}$, cm^{-1}): 3415 (chitin OH str) 3092 (tosyl CH str) 3059 (tosyl CH str) 2958 (chitin CH str) 1927 (tosyl) 1808 (tosyl) 1652 (chitin NH bend) 1592*s* (tosyl) 1538 (chitin amide III) 1488 (tosyl) 1373*s* (tosyl) 1304 (tosyl) 1187 (tosyl) 1174*s* (tosyl) 1120 (tosyl) 1079 (pyranose) 1032 (pyranose) 811*s* (tosyl) 701 (tosyl) 651*s* (tosyl) 604 (chitin) 565*s* (tosyl) 477 (chitin).

2.6. *N-Acetyl-6-tosylchitin* **4**

6-Tosylchitin **3** (82% *DS*, 1.37 g, 4.00 mmol) was stirred in MeOH (30.0 mL) and acetic anhydride (2.0 mL, 2.2 g, 21 mmol) was added. The mixture was stirred overnight and poured into iced water. The reaction mixture was filtered, washed with MeOH, acetone and diethyl ether and dried to give 0.722 g of a yellow solid. The product was stirred in a methanolic 0.1 M KOH solution for 3 h at ambient temperature and filtered. The solid was extensively washed with MeOH, diethyl ether and dried to give 0.561 g of a cream powder **4**.

Calculated for $(\text{chitin})_{0.40}(\text{6-tosylchitin})_{0.60}\cdot 0.8H_2O$: C, 47.2; H, 5.9; N, 4.5; S, 6.2. Found: C, 47.1; H, 6.2; N, 4.4; S, 6.6. IR ($\tilde{\nu}$, cm^{-1}): 3415 (chitin OH str) 2958 (chitin CH str) 1638 (chitin NH bend) 1598 (tosyl) 1540 (chitin amide III) 1450 (tosyl) 1378 (tosyl) 1309 (tosyl) 1187 (tosyl) 1174 (tosyl) 1073 (pyranose) 1032 (pyranose) 969 (chitin) 815 (tosyl) 651 (tosyl) 553 (tosyl).

2.7. *6-Deoxy-6-thioacetylchitin* **5**

N-Acetyl-6-tosylchitin **4** (60% *DS*, 0.453 g, 1.32 mmol) was stirred in DMSO (20.0 mL) and $KSCOCH_3$ (0.411 g, 3.60 mmol) was added. The mixture was stirred at 80 °C overnight under a nitrogen atmosphere and MeOH was added to the cloudy brown solution. The heterogeneous mixture was centrifuged and the brown supernatant liquid discarded. The solid was filtered and washed thoroughly with MeOH, diethyl ether and dried to give 0.285 g of a red-brown solid **5**.

Calculated for $(\text{chitin})_{0.30}(\text{6-thioacetylchitin})_{0.70}\cdot 1.1H_2O$: C, 42.8; H, 6.3; N, 5.3; S, 8.5. Found: C, 43.0; H, 5.9; N, 5.2; S, 8.4. IR ($\tilde{\nu}$, cm^{-1}): 3415 (chitin OH str) 2932 (chitin CH str) 1638 (chitin NH bend) 1618 (chitin NH bend) 1558, 1429, 1377, 1319, 1154, 1115, 1073 (pyranose) 1027 (pyranose) 921 (chitin) 804, 616, 469.

2.8. *6-Deoxy-6-mercaptochitin* **6**

The derivative **5** (70% *DS*, 0.186 g, 0.707 mmol) was stirred in MeOH (5.0 mL) for 30 min at ambient temperature under a nitrogen atmosphere. The reagent NaOMe (95.5 mg, 1.77 mmol) was added and the reaction mixture stirred overnight. The heterogeneous mixture was diluted with water and centrifuged. The solid was washed with distilled water and MeOH and air-dried. The pink-grey solid was further dried under vacuum overnight to give 0.118 g of a brown solid **6**.

Calculated for $(\text{chitin})_{0.45}(\text{6-mercaptochitin})_{0.55}$: C, 45.3; H, 6.2; N, 6.6; S, 8.3. Found: C, 45.2; H, 6.0; N, 6.3; S, 8.1. IR ($\tilde{\nu}$, cm^{-1}): 3416 (OH str) 2955 (CH str) 2600*w* (SH str) 1640 (NH bend) 1557 (amide III) 1377, 1320, 1154 (pyranose) 1117 (pyranose) 1073 (pyranose) 1027 (pyranose) 921, 804, 664, 557.

2.9. *6-Tosylchitosan* **7**

β-Chitosan **2** (80% *DD*, 5.36 g, 29.2 mmol) was stirred in 10.0 M NaOH(aq) solution (110 mL) under vacuum for 2.5 h. Crushed ice (253 g) was added and the mixture stirred until the ice melted. The thick viscous solution was cooled in an ice bath and a solution of TsCl (70.0 g, 367 mmol, 12.5 mol eq.) in $CHCl_3$ (200 mL) was added. The heterogeneous mixture was stirred for 2 h in an ice bath and 2 h in a water bath at ambient temperature. The reaction mixture was poured into distilled water and the supernatant liquid was removed from the fluffy precipitate. Water was repeatedly added to the solid and stirred before removal of the supernatant liquid. The precipitate was filtered, washed with MeOH, diethyl ether and left overnight under vacuum to give 5.84 g of a cream solid **7**.

Calculated for $(\text{chitin})_{0.10}(\text{chitosan})_{0.60}(\text{6-tosylchitin})_{0.30}\cdot 1.1H_2O$: C, 43.1; H, 6.6; N, 6.1; S, 4.2. Found: C, 43.2; H, 6.7; N, 5.6; S, 4.3. IR ($\tilde{\nu}$, cm^{-1}): 3431 (OH str) 2922 (CH str) 1636 (NH bend) 1598 (tosyl) 1541 (amide III) 1497, 1457, 1363, 1176 (tosyl) 1094 (pyranose) 814 (tosyl) 667, 552. Solid-state ^{13}C NMR (δ, ppm): 22.0 (*N*-acetyl CH_3) 57.4 (C2) 61.0 (C6) 75.4 (C3 and C5) 81.6 (C4) 104.9 (C1) 130.0 (tosyl) 137.5 (tosyl) 144.5 (tosyl) 174.3*w* (C=O) 228.4.

2.10. *6-Deoxy-6-thioacetylchitosan* **8**

The compound 6-tosylchitosan **7** (30% *DS*, 5.52 g, 22.2 mmol) was stirred in DMSO (200 mL) for 20 min. The reagent $KSCOCH_3$ (7.60 g, 66.5 mmol) was added and the green mixture was stirred for 16 h under an argon atmosphere at 70 °C. The resulting dark brown solution was poured into MeOH (400 mL) and the precipitate filtered off. The solid was washed thoroughly with MeOH and diethyl ether, air-dried and further dried under vacuum overnight to give 5.23 g of a fawn solid **8**.

Calculated for $(\text{chitin})_{0.15}(\text{6-thioacetylchitin})_{0.33}(\text{6-thioacetylchitosan})_{0.52}\cdot 1.2H_2O$: C, 41.2; H, 6.4; N, 5.5; S, 10.8. Found: C, 41.2; H, 5.8; N, 5.4; S, 11.0. IR ($\tilde{\nu}$, cm^{-1}): 3417 (OH str) 2923 (CH str) 2853 (CH str) 2128, 1735 (S—C=O str) 1638 (NH bend) 1618 (NH bend) 1376, 1323, 1126 (pyranose) 1000 (pyranose) 669, 618, 472. Solid-state ^{13}C NMR (δ, ppm): 23.6 (*N*-acetyl CH_3) 31.2 (*S*-acetyl CH_3) 57.9 (C2) 61.2 (C6) 75.4 (C3 and C5) 83.2 (C4) 105.2 (C1) 129.9 (tosyl) 139.8 (tosyl) 144.2 (tosyl) 175.0*w* (C=O).

2.11. *6-Deoxy-6-mercaptochitosan* **9**

The compound **8** (85% *DS*, 4.89 g, 19.4 mmol) was stirred in MeOH (100.0 mL) for 20 min and NaOMe (2.07 g, 38.8 mmol) was added. The heterogeneous mixture was stirred overnight under a nitrogen atmosphere at ambient temperature. The mixture was filtered and the brown precipitate was washed extensively with distilled water then MeOH. The solid was air-dried and left overnight under vacuum to give 4.54 g of a brown solid **9**.

Calculated for $(\text{chitin})_{0.25}(\text{chitosan})_{0.35}(\text{6-tosylchitin})_{0.10}(\text{6-mercaptochitin})_{0.40}\cdot 0.5H_2O$: C, 44.1; H, 6.4; N, 6.3; S, 7.2. Found: C, 44.1; H, 6.4; N, 6.1; S, 7.4. 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. Solid-state ^{13}C NMR (δ, ppm): 23.1*w* (*N*-acetyl CH_3) 58.0 (C2) 60.8 (C6) 75.4 (C3 and C5) 81.6 (C4) 104.8 (C1) 130.2*w* (tosyl) 173.9*w* (C=O).

A portion of the product **9** (40% *DS*, 250 mg) was stirred in DCl/D_2O solution (p*D* = 2, 10.0 mL) in a capped 10 mL conical flask at 70 °C to form a yellow gel which contained yellow lumps. A solution of $NaNO_2$ (72.0 mg, 1.0 mmol) in D_2O (1.0 mL) was added. Nitrogen gas was initially given off and the yellow solution and yellow lumps were stirred for 45 min. The reaction mixture was cooled to ambient temperature and the solution was decanted from the solid. The solution was filtered (0.2 μm filter) and stored at 2 °C.

^{1}H NMR (δ, ppm, DCl/D_2O): 1.8–2.5, 3.1–5.4, 6.07*w*, 7.2*w*–9.7*w*. ^{13}C NMR (δ, ppm, DCl/D_2O): 22.29, 61.13, 76.75, 77.64, 83.12, 84.47, 89.89, 130*w*, 171*w*, 175*w*, 190*w*. HRMS (+ESI mode): *m/z* calculated for [M$^+$] (found): $[C_8H_{12}O_4S]^+$ 204.046 (204.062); $[C_{10}H_{14}O_5S]^+$ 246.056 (245.010); $[C_{14}H_{22}NO_9]^+$ 348.137 (348.105); $[C_{16}H_{25}NO_9S]^+$ 407.125 (407.141); $[C_{18}H_{27}NO_{10}S+H]^+$ 449.136 (449.182); $[C_{21}H_{29}NO_{10}S_2]^+$ 519.123 (519.138); $[C_{23}H_{31}NO_{11}S_2]^+$ 561.134 (561.180).

2.12. *6-Mercaptochitin-graft-poly(MMA)* **10**

The product **6** (20% *DS*, 50.3 mg) was stirred in DMSO (4.0 mL) under vacuum for 2 h and MMA (4.0 mL, 37 mmol) was added.

The reaction mixture was stirred at 90 °C for 45 h. The yellow gel was poured into MeOH and the gel-like lumps were placed into acetone (200 mL) and stirred for 72 h. The mixture was filtered and the white solid was stirred in acetone (400 mL) overnight then filtered. The filtered solid was washed with acetone, air-dried and left under vacuum overnight to give 93.4 mg of the cream solid **10**.

Calculated for $(\text{polysaccharide})_{1.0}\cdot(\text{pMMA})_{18.0}$: C, 58.3; H, 7.9; N, 0.7; S, 0.3. Found: C, 58.2; H, 8.1; N, 0.4; S, 0.0. IR ($\tilde{\nu}$, cm^{-1}): 3455 (chitin OH str) 3002 (CH str) 2953 (CH str) 1732 (pMMA C=O str) 1636 (chitin NH bend) 1487 (CH bend) 1453 (CH bend) 1387 (pMMA) 1274 (pMMA) 1245 (CO str) 1195 (CO str) 1149 (CO str) 989 (pMMA) 750 (CH rock). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.84 and 1.02 (2× s, 3H, pMMA C**H**$_3$) 1.85 (s, 2H, pMMA C**H**$_2$) 3.59 (s, 3H, pMMA ROC**H**$_3$).

The combined acetone filtrates were reduced in volume and poured into MeOH. The white fibrous rubbery solid was filtered and washed with MeOH then dried under vacuum overnight to give 1.96 g of a white solid (homopolymer).

Calculated for pMMA: C, 60.0; H, 8.0. Found, C, 59.4; H, 7.9. IR ($\tilde{\nu}$ cm^{-1}): 3455 (water OH str) 3002 (CH str) 2954 (CH str) 1732 (C=O str) 1636 (water OH bend) 1485 (CH bend) 1450 (CH bend) 1389 (pMMA) 1273 (pMMA) 1244 (CO str) 1194 (CO str) 1149 (CO str) 1063*w* (pMMA) 990 (pMMA) 841 (pMMA) 751 (CH rock). ^{1}H NMR (δ, ppm, $CDCl_3$): 0.84 and 1.02 (2× s, 3H, C**H**$_3$) 1.85 (s, 2H, C**H**$_2$) 3.59, (s, 3H, ROC**H**$_3$).

2.13. 6-Mercaptochitosan-graft-poly(MMA) **11**

The product **9** (15% *DS*, 49.1 mg, 0.25 mmol) was stirred in DMSO (3 mL) under reduced pressure for 2 h. The reagent MMA (10.0 mL, 93.5 mmol) was added and the mixture was stirred at 95 °C for 21 h to form a clear solid with yellow lumps. The heterogeneous solid was stirred overnight in a large amount of acetone. The resulting brown solid was stirred in acetone overnight a second time and the suspension filtered. The resulting brown solid was extensively washed with acetone and air-dried to give 69.8 mg of a light brown solid **11**.

Calculated for $(\text{polysaccharide})_{1.0}\cdot(\text{pMMA})_{2.2}$: C, 51.8; H, 7.3; N, 3.2; S, 2.2. Found: C, 51.3; H, 7.5; N, 2.9; S, 1.0. IR ($\tilde{\nu}$, cm^{-1}): 3416*s* (chitin OH str) 3001 (CH str) 2953 (CH str) 1732 (C=O str) 1638*m* (chitin NH bend) 1618*m* (chitin NH bend) 1451 (CH bend) 1387 (pMMA) 1273 (pMMA) 1243 (pMMA CO str) 1194 (pMMA CO str) 1150 (pMMA CO str) 1062 (chitin pyranose) 993 (pMMA) 750 (CH rock).

The combined acetone washings were reduced in volume and poured into MeOH. The resulting white suspension was filtered, the solid was washed with MeOH and air-dried to give 6.76 g of a fluffy white solid (homopolymer).

2.14. 6-Mercaptochitosan-graft-poly(MMA) in acetic acid solution **12**

The polymer **9** (40% *DS*, 30.1 mg) was stirred in 0.5 M AcOH(aq) solution (5.0 mL) at ambient temperature to form a gel. MMA (4.6 mL, 43 mmol) was added and the mixture was stirred at 85 °C for 48 h. Additional solvent (5.0 mL) was added after 24 h. The resulting mixture was stirred in acetone (400 mL) for 48 h and filtered. Fresh acetone was added to the solid and the mixture was stirred for a further 48 h. The suspension was filtered and the solid washed with acetone. The solid was left overnight under vacuum and ground up to give 198 mg of a lumpy yellow solid **12c**.

Calculated for $(\text{polysaccharide})_{1.0}\cdot(\text{pMMA})_{29.0}$: C, 58.8; H, 7.9; N, 0.4; S, 0.5. Found: C, 58.5; H, 8.0; N, 0.4; S, 0.0. IR ($\tilde{\nu}$, cm^{-1}): 3474*s* (OH str) 3007 (CH str) 2956 (CH str) 1732*br* (C=O str) 1638 (chitin NH bend) 1618 (chitin NH bend) 1488*br* (CH bend) 1455*br* (CH bend) 1386 (pMMA) 1274*br* (pMMA) 1245*br* (CO str) 1197*br* (CO str) 1149*br* (CO str) 1065 shoulder (chitin) 988 (pMMA) 750 (CH rock).

The combined acetone washings were reduced under vacuum and precipitated in MeOH. The white solid was filtered, air-dried and dried under vacuum for two nights to give 4.98 g of a white solid (homopolymer).

3. Results and discussion

β-Chitin and β-chitosan were reacted with tosyl chloride and the resulting tosylated polysaccharides were reacted with potassium thioacetate and sodium methoxide to form 6-mercaptochitin and mercaptochitosan, respectively. These thiolated polymers were subsequently graft polymerised with methyl methacrylate to form grafted copolymers. The solvent had an influence on the success of the polymerisation with the chitosan polymers giving highly grafted materials in aqueous acetic acid solution.

3.1. Tosylation

Since the reactivity of the polymorphs of chitin can differ β-chitin extracted from squid pens was tosylated by the method reported by Kurita for α-chitin from shrimp shells (Kurita et al., 1992). The polymer β-chitin was reacted with tosyl chloride in a heterogeneous reaction under basic conditions in a two-phase water/chloroform system with a large molar excess of tosyl chloride (Fig. 1). The products obtained from β-chitin by this method were found to have a wide range of degrees of tosylation as determined by elemental analysis (Table 1). The amount of tosylation varied from a monotosylation of 50% of the sugars in the chain to three tosyl groups on every sugar in the chain. The products obtained from β-chitin in the present work showed a variation in the degree of tosylation larger than that reported by Kurita for α-chitin (Kurita et al., 1992). This variability in the amount of tosylation was attributed to the enhanced reactivity of β-chitin over α-chitin due to the weaker intermolecular hydrogen bonding between the chitin chains that occurs in β-chitin (Roberts, 1992). Further disruption to the hydrogen bonding may have resulted from the presence of the large tosyl groups on the polymer chains.

The microanalytical data of the products were consistent with the tosylated polymers still containing some free amines in the backbone. The presence of three types of sugars in the polymer made the characterisation more involved than for a polymer with two sugar units. Therefore the products were treated with acetic anhydride to acetylate the amines and then washed with a weak basic solution of 0.1 M KOH in MeOH to remove any acetyl groups on the oxygen atoms to give fully *N*-acetylated chitin derivatives **4** (Fig. 1).

The microanalytical results of the *N*-acetylated polymers **4** (Table 1) suggested that the amount of tosylation was lower after treatment of the polymer **3** with acetic anhydride and washing with weak base. The mild base used under these reaction conditions may have been responsible for replacing the tosyl groups which are known to be good leaving groups. The disappearance of the acetyl peak in the IR spectrum at 1755 cm^{-1} after the base wash indicated that the acetyl groups on the oxygen atoms at the C2 and C6 positions were easily removed leaving only acetamide groups in the polymer **4**. The acetamide groups were not affected by these mild reaction conditions as this functional group is very stable (Brown & Foote, 1998). This high reactivity was again attributed to the open structure of the polymer derived from β-chitin. The acetic anhydride reacted with both the amines and the hydroxyl groups and the treatment with base removed the acetate groups from the hydroxyls.

Table 1
The products 6-tosylchitin **3** and *N*-acetyl-6-tosylchitin polymers **4** formed by the tosylation of β-chitin.

Sample	DS[a] (%)	Formula[b]	Calculated (%) Found[c] (%)			
			C	H	N	S
3	50	$(\text{CH})_{0.32}(\text{CS})_{0.18}(\text{OTs-CH})_{0.50}\cdot 1.6\text{H}_2\text{O}$	44.4 *44.3*	6.3 *5.9*	4.6 *4.7*	5.3 *5.0*
3	60	$(\text{CH})_{0.22}(\text{CS})_{0.18}(\text{OTs-CH})_{0.60}\cdot 2.1\text{H}_2\text{O}$	43.6 *43.5*	6.3 *5.5*	4.3 *4.3*	5.9 *6.1*
4	30	$(\text{CH})_{0.70}(\text{OTs-CH})_{0.30}\cdot 0.9\text{H}_2\text{O}$	45.7 *45.5*	6.3 *5.7*	5.3 *5.4*	3.6 *3.7*
3	82	$(\text{CS})_{0.18}(\text{OTs-CH})_{0.82}\cdot 1.2\text{H}_2\text{O}$	46.8 *46.8*	5.9 *5.8*	4.1 *4.0*	7.7 *7.8*
4	60	$(\text{CH})_{0.40}(\text{OTs-CH})_{0.60}\cdot 0.8\text{H}_2\text{O}$	47.2 *47.1*	5.9 *6.2*	4.5 *4.4*	6.2 *6.6*
3	120	$(\text{OTs-CH})_{0.80}(2\text{OTs-CH})_{0.20}\cdot 0.8\text{H}_2\text{O}$	48.9 *48.7*	5.5 *5.6*	3.5 *3.5*	9.6 *9.9*
4	90	$(\text{CH})_{0.10}(\text{OTs-CH})_{0.90}$	50.1 *50.0*	5.4 *5.5*	4.2 *4.2*	8.6 *8.5*
3	220	$(2\text{OTs-CH})_{0.80}(\text{NTs-2OTs-CH})_{0.20}\cdot 2.5\text{H}_2\text{O}$	47.7 *47.3*	5.4 *5.1*	2.4 *2.3*	12.2 *12.6*
3	300	$(\text{NTs-2OTs-CH})_{1.00}\cdot 6.4\text{H}_2\text{O}$	43.9 *43.6*	5.7 *4.7*	1.9 *1.3*	13.0 *13.6*
4	25	$(\text{CH})_{0.75}(\text{OTs-CH})_{0.25}\cdot 0.9\text{H}_2\text{O}$	45.4 *45.4*	6.4 *6.6*	5.4 *5.6*	3.1 *3.0*

[a] A *DS* of 100% indicates that one tosyl group is bound to every sugar.
[b] The abbreviations, description of the sugars and molecular formulae: CH [chitin ($C_8H_{13}NO_5$)]; CS [chitosan ($C_6H_{11}NO_4$)]; OTs-CH [6-tosylchitin ($C_{15}H_{19}NO_7S$)]; 2OTs-CH [3,6-ditosylchitin ($C_{22}H_{25}NO_9S_2$)]; NTs-2OTs-CH [2-tosylamine-3,6-ditosylchitin ($C_{27}H_{29}NO_{10}S_3$)].
[c] Found values are given in italics.

The Kurita method was also used to tosylate β-chitosan (Kurita et al., 1992). The two products contained sulfur and the microanalytical data were consistent with a degree of tosylation of 30% and 20%, respectively. The *DS* of tosyl groups was much lower for the products prepared from chitosan than from chitin but this tosylation route was still considered successful and these polymers were subsequently reacted to form thiolated chitosan derivatives.

3.2. Thiolation

The polymer *N*-acetyl-6-tosylchitin **4** was reacted with potassium thioacetate to replace the tosyl groups giving 6-deoxy-6-thioacetylchitin **5**. The insoluble polymer was subsequently treated with sodium methoxide to remove the acetyl groups to obtain the product 6-deoxy-6-mercaptochitin **6** (Fig. 1).

Two of the fully *N*-acetylated 6-tosylchitin samples **4** were used with *DS* values of the tosyl groups of 30% and 60%, respectively. These two samples were selected as it was expected that these *DS* values would be retained throughout the reaction sequence giving products with two different ratios of the thiol functional group. Although the *DS* values of the thioacetylated polymers were higher than expected, the *DS* values of the thiolated polymers were similar to the *DS* values of the 6-tosylchitin samples (Table 2). The incorporation of the thiol groups into β-chitin was achieved and meant this synthetic route to prepare 6-deoxy-6-mercaptochitin from β-chitin was considered successful. The microanalytical data

Table 2
The *DS* and microanalytical data for the series of products **5** and **6** prepared from 6-tosylchitin **4**.

Sample	Reaction role	DS (%)	Formula[a]	Calculated (%) Found[b] (%)			
				C	H	N	S
4	Reagent	30	$(\text{CH})_{0.70}(\text{OTs-CH})_{0.30}\cdot 0.9\text{H}_2\text{O}$	45.7 *45.5*	6.3 *5.7*	5.3 *5.4*	3.6 *3.7*
5	Intermediate	90	$(\text{CH})_{0.10}(\text{SAc-CH})_{0.90}\cdot 3.6\text{H}_2\text{O}$	36.7 *35.2*	6.9 *4.9*	4.4 *4.6*	9.0 *8.8*
6	Product	20	$(\text{CH})_{0.80}(\text{SH-CH})_{0.20}\cdot 0.5\text{H}_2\text{O}$	44.6 *44.7*	6.6 *7.0*	6.5 *6.3*	3.0 *2.7*
6	Product	25	$(\text{CH})_{0.75}(\text{SH-CH})_{0.25}\cdot 0.3\text{H}_2\text{O}$	45.2 *45.4*	6.4 *6.4*	6.6 *6.4*	3.8 *3.5*
4	Reagent	60	$(\text{CH})_{0.40}(\text{OTs-CH})_{0.60}\cdot 0.8\text{H}_2\text{O}$	47.2 *47.1*	5.9 *6.2*	4.5 *4.4*	6.2 *6.6*
5	Intermediate	70	$(\text{CH})_{0.30}(\text{SAc-CH})_{0.70}\cdot 1.1\text{H}_2\text{O}$	42.8 *43.0*	6.3 *5.9*	5.3 *5.2*	8.5 *8.4*
6	Product	55	$(\text{CH})_{0.45}(\text{SH-CH})_{0.55}$	45.3 *45.2*	6.2 *6.0*	6.6 *6.3*	8.3 *8.1*

[a] The abbreviations, description of the sugars and molecular formulae: CH [Chitin ($C_8H_{13}NO_5$)]; OTs-CH [6-tosylchitin ($C_{15}H_{19}NO_7S$)]; SAc-CH [6-thioacetylchitin ($C_{10}H_{15}NO_5S$)]; SH-CH [6-mercaptochitin ($C_8H_{13}NO_4S$)].
[b] Found values are given in italics.

were consistent with all the sugars containing an acetamide group instead of a free amine at the C2 position. The acetamide groups were retained under the reaction conditions used. This acetylation was consistent with a side reaction of thioacetate to form stable acetamide groups in the polymer (Madaj, Trynda, Jankowska, & Wisniewski, 2002; Whistler, Anisuzzaman, & Kim, 1973) especially as there was an excess of potassium thioacetate in the reaction mixture. The IR spectra of the products provided evidence for the reactions of 6-tosylchitin to form the product **6**. The spectrum of the polymer **6** displayed a weak peak at approximately 2600 cm^{-1} assigned to the SH stretch. The IR spectrum of 6-mercaptochitin prepared by Kurita (Kurita, Yoshino, Nishimura, & Ishii, 1993) agreed well with the spectra of the 6-mercaptochitin products prepared in the present work.

The polymer 6-tosylchitosan **7** was reacted with potassium thioacetate to form 6-thioacetylchitosan **8** which was subsequently deacetylated with sodium methoxide to give the product 6-mercaptochitosan **9**. The products were analysed by microanalyses to give estimates of the various sugar units and *DS* values of the sulfur-containing groups (Table 3). Three of the samples of **9** had *DS* values of the thiol group of 15% and one sample had a higher *DS* value of 40%. This more substituted sample was investigated more thoroughly due to the potential differences in properties arising from the higher amount of thiol groups. The microanalytical data for the polymers **8** and **9**, prepared from the tosylated chitosan polymer **7**, suggested an increase in the number of acetyl groups on the polymers after both reactions, which was similar to the reactivity of tosyl chitin under the same reaction conditions. Acetyl groups are labile and can migrate from their original positions (Madaj et al., 2002). It was proposed that a portion of the acetyl groups on the sulfur atoms underwent migration to the other heteroatoms, oxygen and nitrogen, in the polymer (Whistler et al., 1973). During the treatment with sodium methoxide, the stable acetamide groups were not removed and the resulting polymers had higher degrees of *N*-acetylation.

The IR spectra of chitosan **2** and the resulting sulfur-containing polymers **7**, **8** and **9** are shown in Fig. 2. The spectrum of **7** displayed significant peaks assigned to the tosyl group as well as those expected for chitosan. The spectrum of **8** displayed a peak at 1735 cm^{-1} assigned to the S—C═O stretch, which was consistent with the replacement of some tosyl groups by thioacetyl groups. The absence of this peak in the spectrum of **9** showed that the removal of the thioacetyl group was possible under the reaction conditions used. However these qualitative data could not be used to estimate the proportions of the various sugar residues in the polymer samples.

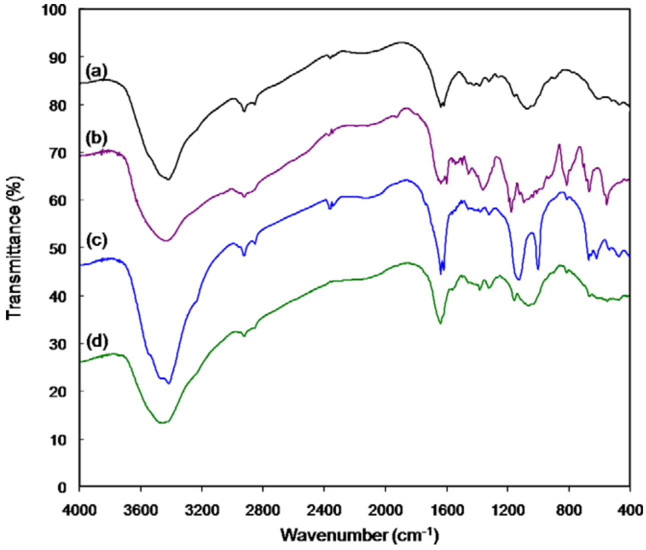

Fig. 2. The IR spectra of (a) chitosan **2** and the sulfur-containing polymers (b) tosylchitosan **7**, (c) thioacetylchitosan **8** and (d) mercaptochitosan **9**. The traces are offset for clarity.

The solid-state ^{13}C NMR spectra are shown in Fig. 3 for the samples **2**, **7**, **8** and **9**. The peaks at approximately 120–150 ppm in the spectrum of polymer **7** were assigned to the tosyl groups. The presence of these peaks in the spectra of **8** and **9** led to the conclusion that the removal of the tosyl groups was incomplete. The presence of a peak at approximately 30 ppm in the spectrum of **8** was as-

Table 3
The proposed sugar ratios in the series of products produced from the 6-tosylchitosan polymers.

Sample	DS[a] (%)	Formula[b]	Calculated (%) Found[c] (%)			
			C	H	N	S
7	30 (OTs)	$(CH)_{0.10}(CS)_{0.60}(OTs\text{-}CH)_{0.30}\cdot 1.1H_2O$	43.1 *43.2*	6.6 *6.7*	6.1 *5.6*	4.2 *4.3*
8	85 (SAc)	$(CH)_{0.15}(SAc\text{-}CH)_{0.33}(SAc\text{-}CS)_{0.52}\cdot 1.2H_2O$	41.2 *41.2*	6.4 *5.8*	5.5 *5.4*	10.8 *11.0*
9	40 (SH)	$(CH)_{0.25}(CS)_{0.25}(OTs\text{-}CH)_{0.10}(SH\text{-}CH)_{0.40}\cdot 0.5H_2O$	44.1 *44.1*	6.4 *6.4*	6.3 *6.1*	7.2 *7.4*
8	25 (SAc)	$(CH)_{0.75}(SAc\text{-}CS)_{0.25}\cdot 0.45H_2O$	44.6 *44.8*	6.5 *7.1*	6.5 *6.0*	3.7 *3.7*
9	15 (SH)	$(CH)_{0.10}(CS)_{0.75}(SH\text{-}CH)_{0.15}\cdot 1.8H_2O$	40.9 *41.1*	7.1 *6.5*	7.7 *6.2*	2.6 *2.5*
9	15 (SH)	$(CH)_{0.10}(CS)_{0.75}(SH\text{-}CS)_{0.15}\cdot 1.3H_2O$	38.9 *39.2*	7.3 *6.6*	7.3 *6.0*	2.5 *2.5*
7	20 (OTs)	$(CH)_{0.10}(CS)_{0.70}(OTs\text{-}CH)_{0.20}\cdot 0.8H_2O$	43.3 *43.5*	6.7 *7.0*	6.7 *6.4*	3.1 *2.7*
8	15 (SAc)	$(CH)_{0.10}(CS)_{0.75}(SAc\text{-}CS)_{0.15}\cdot 0.4H_2O$	43.1 *43.5*	6.8 *7.1*	7.7 *6.4*	2.7 *2.2*
9	15 (SH)	$(CH)_{0.10}(CS)_{0.75}(SH\text{-}CS)_{0.15}\cdot 0.1H_2O$	43.9 *44.1*	6.8 *6.8*	8.3 *7.2*	2.8 *2.2*

[a] The *DS* is given for the added functional group in the polymer.

[b] The abbreviations and description of the sugars: CH [chitin]; CS [chitosan]; OTs-CH [6-tosylchitin]; SAc-CH [6-thioacetylchitin]; SH-CH [6-mercaptochitin]; SAc-CS [6-thioacetylchitosan]; SH-CS [6-mercaptochitosan].

[c] Found values are given in italics.

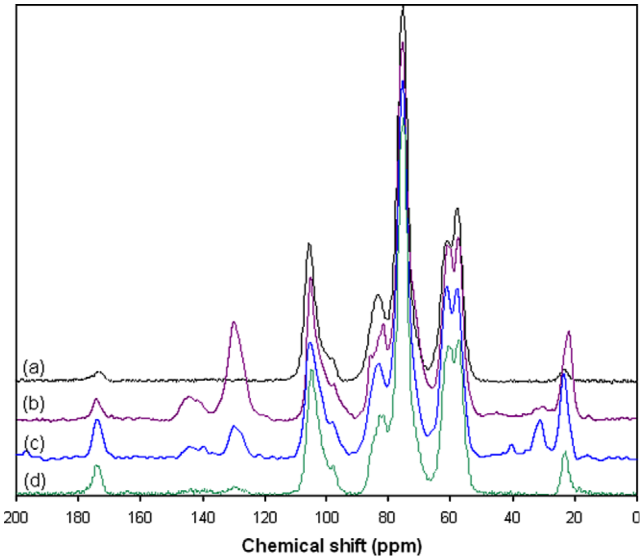

Fig. 3. The solid-state ^{13}C NMR spectra (a) chitosan **2** and the sulfur-containing polymers (b) tosylchitosan **7**, (c) thioacetylchitosan **8** and (d) mercaptochitosan **9**. The traces are offset for clarity. The major points of interest in the spectra were the tosyl peaks from approximately 120–150 ppm which remain in the products **8** and **9** and the changing relative intensities of the acetamide peaks at approximately 20 and 175 ppm.

signed to the thioacetyl group. This peak was absent in the spectrum of **9** which suggested that the acetyl groups were removed from the sulfur by the basic methoxide used in the reaction. This was consistent with the data from IR spectroscopy for this product. The peaks at approximately 20 and 175 ppm were assigned to the carbons in the acetamide group. The increased intensities of these peaks in the spectrum of **8** compared to the spectrum of **2** were consistent with *N*-acetylation during the thioacetylation of the tosylated polymer. The higher intensities of the acetamide peaks in the final polymer **9** compared to **2** provided evidence for the incomplete deacetylation under the reaction conditions used. These data also provided strong evidence that the nitrogen remained acetylated after the conversion of thioacetyl groups to thiol groups. This was consistent with the acetylation behaviour described earlier for the polymers **4** and **6**.

The solid-state ^{13}C NMR spectra suggested the presence of tosyl groups in the polymer. However, the amounts of these groups were not quantifiable from the acquired spectrum. The insoluble polymer **9** with *DS* = 40% was degraded with sodium nitrite to give soluble oligomers in an attempt to identify the sugar residues in the polymer by mass spectrometry. The calculated *m/z* ratios of possible oligomers were compared to the *m/z* peaks in the spectrum to give a number of proposed oligomers that were produced. Two peaks at *m/z* = 519.138 and 561.180 in the mass spectrum were assigned to a tosylated disaccharide unit with or without an acetyl group, respectively. These peaks provided more evidence for the incomplete substitution of the tosyl group by thioacetate. The mass spectrometry data supported the partial deacetylation of thioacetyl groups in the polymer to thiol groups, with a corresponding increase in the amount of acetyl groups on the nitrogen atoms, which was suggested by the solid-state ^{13}C NMR data. Incorporation of a small number of tosyl groups in the calculation of the microanalytical data gave the closest fit between the calculated and experimentally determined mass values.

The polymer **9** displayed a high degree of swelling and rapidly formed a gel in the solvent 0.3 M AcOH/0.2 M AcONa(aq). However, it was insoluble in DMSO, DMF, acetonitrile, water, 0.5 M AcOH solution and 0.5 M phosphate buffer. This high degree of swelling was significant as the polymer **6** was not swollen under the same conditions.

3.3. Graft polymerisation

The sulfur-containing β-chitin and β-chitosan derivatives were used to prepare a series of grafted chitin-*graft*-poly(MMA) and chitosan-*graft*-poly(MMA) derivatives. Kurita et al. have reported the successful polymerisation of grafted chitin copolymers using 6-mercaptochitin from α-chitin as the trunk polymer (Kurita, Hashimoto, Ishii et al., 1996; Kurita, Hashimoto, Yoshino et al., 1996; Kurita et al., 2002). It was proposed that chitin copolymers could also be formed from related polymers prepared here, although these samples displayed lower *DS* values than those prepared by Kurita. MMA was investigated as the alkene groups were capable of reacting by a free-radical mechanism with the thiolated polysaccharides and the ester side-chain can be modified by hydrolysis after polymerisation to give carboxylic acid groups instead of ester groups (Kurita et al., 2002) which alters the solubility of the polymers.

Two samples of 6-mercaptochitin **6** with differing *DS* values of 20% and 55%, respectively, were used as the different amounts of thiol groups may have affected the degree of grafting. The highly thiolated chitin (*DS* = 55%) gave products with low degrees of grafting in contrast to the less thiolated chitin sample (*DS* = 20%) which gave highly grafted products (Table 4). This difference was attributed to a lower degree of swelling for the more substituted polymer and a corresponding reduction in accessibility to the sulfur radicals by the monomer. The grafted copolymers became slightly soluble in DMSO and $CHCl_3$ as the amount of grafted poly(MMA) increased to high levels. The ^{1}H NMR spectrum of the grafted copolymer was acquired in $CDCl_3$ and all the peaks were assigned to poly(MMA). The low proportion of chitin in the polymer meant that there were no peaks assigned to chitin in the spectrum. All of the grafted polymers were insoluble in acetone, aqueous acetic acid solution and water.

The IR spectra of the grafted products **10** all contained peaks assigned to both the chitin polymer and the synthetic poly(MMA) (Fig. 4). The intensities of the peaks differed with an increase in the amount of grafting. However, a comparison of the polysaccharide and poly(MMA) spectra showed a significant overlap of the peaks. Therefore the degree of grafting was not quantifiable by this technique. The highly grafted sample was analysed by microanaly-

Table 4
The chitin-*graft*-poly(MMA) copolymers **10** prepared from the grafting of MMA with 6-mercaptochitin **6**.

Starting material **6**		Reaction conditions		Product **10**		
DS (%)	Amount (mg)	MMA (mL)	Temperature (°C)	Yield (mg)	Appearance	Grafted[a]
55	23.2	0.90	75	9.2	Pale yellow solid	Slightly
55	27.1	1.4	80–85	8.5	Yellow solid	Slightly
20	50.3	4.0	90	93.4	Cream solid	Highly
20	49.1	4.0	95	10.1	Tan powder	No

[a] The grafting efficiencies were estimated from the IR spectra as there were limited amounts of the samples for microanalysis.

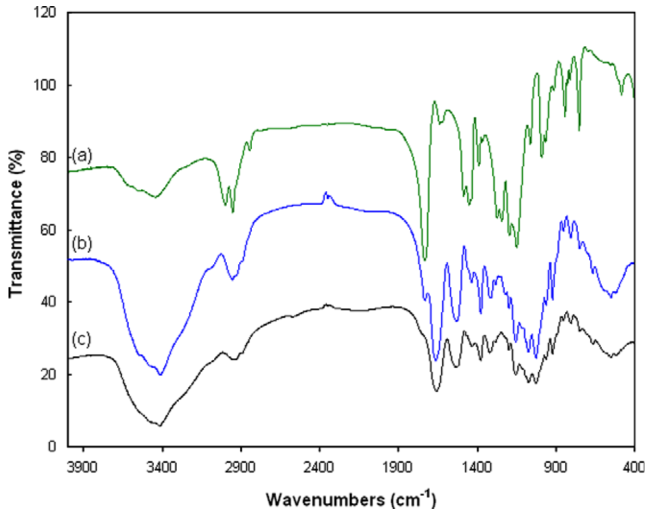

Fig. 4. The IR spectra of (a) poly(MMA) polymers, (b) grafted chitin-*graft*-poly(MMA) **10** and (c) 6-mercaptochitin **6**. The overlapping peaks of the chitin and poly(MMA) polymers meant the degree of grafting of the copolymer was not estimated by this method.

sis and this was consistent with a high degree of grafting of 18 MMA monomer units for each sugar unit. Although the polymerisation reactions with 6-mercaptochitin were successful, the variation in grafting efficiency and the limited solubility of the polymers meant that 6-mercaptochitin polymers prepared from β-chitin may be less reliable than 6-mercaptochitin polymers derived from α-chitin (Kurita, Hashimoto, Ishii et al., 1996).

The swelling of the thiolated polymer 6-mercaptochitosan **9** (prepared from β-chitosan) in acetic acid buffers meant that the resulting graft copolymers of this material may have been more soluble than the chitin based copolymers. The presence of the free amine groups may have influenced the solubility of the grafted polymers. A range of grafted copolymers were prepared with various reaction conditions and the effects of concentration, reaction ratio, temperature and solvent were examined.

The initial reactions of 6-mercaptochitosan **9** were performed in DMSO with differing reaction ratios of the monomer to the polysaccharide. The resulting polymers differed in appearance and yields (Table 5) and the grafted efficiencies were estimated from the microanalytical data. The amounts of grafting were fairly low for the prepared polymers and ranged from 0.7 to 2.2 MMA monomeric units for each sugar. Although two of the derivatives were reasonably grafted the heterogeneous appearance of the solids was undesirable. The poor swelling of the polymer **9** in DMSO may have affected the amount of grafting of the polymer. Therefore an additional reaction was investigated under the same reaction conditions with 0.5 M AcOH in DMSO as the solvent to give the product **11**. No homopolymer was produced and the microanalytical data indicated that there was no polymerisation. The data were consistent with the degradation of the parent polymer at the elevated temperatures used in the reaction. The range of appearances and grafting degrees of the grafted copolymers prepared with **9** and MMA in DMSO led to investigation of another solvent. The high swelling of the polymer **9** in acetic acid solutions and buffers meant an aqueous acetic acid solution was investigated for the grafting reaction. The reaction conditions and results are shown in Table 6. A polymerisation of MMA was also investigated under the same reaction conditions to form the homopolymer poly(MMA). The higher yields of grafted polymers and the consistency in appearance of the polymers prepared in acetic acid solution suggested this solvent was preferable to DMSO. Although the reaction was still heterogeneous the higher swelling of the chitosan polymers and the higher accessibility of the thiol groups in aqueous

Table 5
The grafted polymers **11** prepared from **9** and MMA in DMSO.

Starting material **9**		Reaction conditions		Product **11**			
DS (%)	Amount (mg)	MMA (mL)	Temperature (°C)	Yield (mg)	Appearance	Grafted[a]	Grafted[b] (mol eq.)
40	58.4	1.5	95	51.0	Orange solid	Moderately	0.7
40	58.5	3.0	95	80.6	Brown/white solid[c]	Moderately	1.5
40	58.8	6.0	95	89.0	Light brown/white solid[c]	Moderately	1.8
15	49.1	10.0	95	69.8	Light brown solid	Moderately	2.2
15	26.3	1.85	95	25.1	Yellow powder	Moderately	0.7
15	50.5	3.5	95	49.1	Red solid	No	–
15	25.5	3.0	95	18.8	Orange solid	No	–
15	20.6	2.5	95	9.3	Red solid	No	–
40	49.9	1.6	85	43.0	Yellow solid	No	–
40[d]	49.7[d]	1.6[d]	85[d]	41.7[d]	Red solid[d]	No	–

[a] The grafting efficiencies as determined by IR spectroscopy.
[b] The grafting efficiencies as determined by elemental analysis, in molar equivalents per sugar unit of the polysaccharide.
[c] The solid was heterogeneous.
[d] The reaction was performed in 0.5 M AcOH in DMSO.

Table 6
The grafted polymers **12** prepared in 0.5 M AcOH(aq) solution from **9** (*DS* = 40%).

9 (mg)	[9] (g mL^{-1})	MMA (mL)	Ratio[a] (1:X)	Yield (mg)	Appearance	Grafted[b]	Grafted[c] (mol eq.)	Grafted[d] (mol eq.)[c]
30.3	6	1.15	75	62.4	Yellow powder	Highly	18.0	12.7
30.0	6	2.3	150	19.2	Yellow powder	No	–	–
30.1	6	4.6	300	197.5	Yellow solid	Highly	29.0	19.1
15.0	3	2.3	300	112.4	Yellow solid	Highly	50.0	36.0
45.0	9	6.9	300	445.4	Yellow solid	Highly	30.0	28.5
–	–	2.3	–	1530.0	White powder	Poly(MMA)	–	–

[a] The ratio is the number of molar equivalents of monomer compared to each sugar equivalent.
[b] The grafting efficiencies as determined by IR spectroscopy.
[c] The grafting efficiencies as determined by elemental analysis, in molar equivalents per sugar unit of the polysaccharide.
[d] The grafting efficiencies as determined by TGA, in molar equivalents per sugar unit of the polysaccharide.

acidic conditions made the reaction proceed more smoothly and less variation between copolymer samples than those prepared in DMSO.

The IR spectra of all but one of the products **12** were consistent with a high degree of grafting and showed similarities to the spectra of the thiolated polymer **9** and the homopolymer poly(MMA). The microanalytical data for the polymers were suggestive of a high proportion of grafting. The results were consistent with a degree of grafting from 18 to 50 MMA units for each sugar unit.

4. Conclusion

Chitin and chitosan were prepared from squid pens and reacted with tosyl chloride to form 6-tosylchitin and 6-tosylchitosan, respectively. The amount of tosylation in 6-tosylchitin prepared from β-chitin varied to a greater extend than previously reported for α-chitin and this difference was attributed to an enhanced reactivity of β-chitin over α-chitin due to the weaker intermolecular hydrogen bonding between the chitin chains. Further reaction of 6-tosylchitin and 6-tosylchitosan with potassium thioacetate and sodium methoxide gave 6-mercaptochitin and 6-mercaptochitosan, respectively, and these derivatives were characterised by microanalysis, IR and solid-state ^{13}C NMR spectroscopy. The polymer 6-mercaptochitosan retained tosyl groups even after further reaction and showed lower degrees of substitution than 6-mercaptochtin.

Both polymers were reacted with MMA to form 6-mercaptochitin-*graft*-poly(MMA) and 6-mercaptochitosan-*graft*-poly(MMA). The obtained grafted copolymers were characterised by microanalysis and IR spectroscopy. The grafted copolymers prepared with 6-mercaptochitosan showed higher levels of poly(MMA) and were more homogeneous in appearance. The solvent had an influence on the degree of grafting with the largest amount of poly(MMA) present in the copolymers prepared in aqueous acetic acid solution.

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