Polymer Chemistry

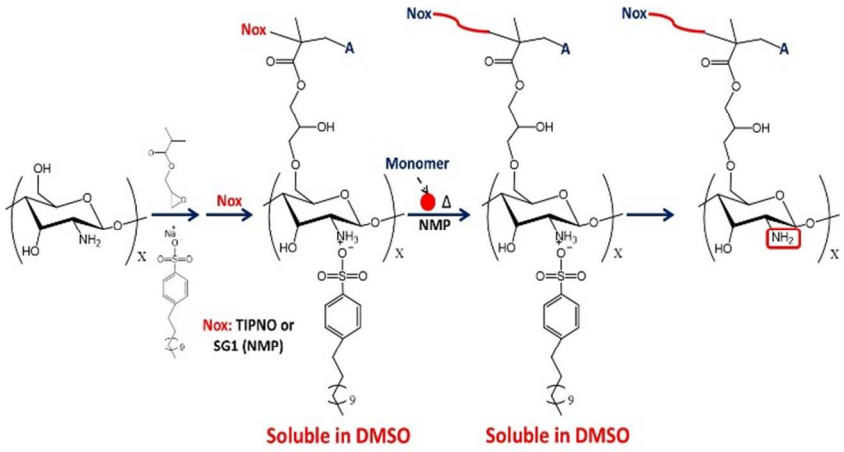
Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/polymers

Modification of chitosan with polystyrene and poly(n-butyl acrylate) via nitroxide-mediated polymerization and *grafting from* approach in homogeneous media

O. García-Valdez, R. Champagne-Hartley,[a] E. Saldívar-Guerra, P. Champagne, and M. F. Cunningham



Nitroxide-mediated polymerization was used to graft modify solubilized chitosan, allowing the reaction to be performed homogeneously.

Journal Name

RSCPublishing

ARTICLE

Modification of chitosan with polystyrene and poly(n-butyl acrylate) via nitroxide-mediated polymerization and *grafting from* approach in homogeneous media

Cite this: DOI: 10.1039/x0xx00000x

Received ooth January 2012,
Accepted ooth January 2012

DOI: 10.1039/x0xx00000x

www.rsc.org/

O. García-Valdez,[a,b] R. Champagne-Hartley,[a] E. Saldívar-Guerra,[b*] P. Champagne,[a,c] and M. F. Cunningham[a*]

Chitosan (CTS) modification with polystyrene (PS) and poly(n-butyl acrylate) (PnBA) via nitroxide-mediated polymerization (NMP) and a grafting from approach is reported. CTS was first functionalized with glycidyl methacrylate (GMA) and then converted into a macroalkoxyamine by intermolecular 1,2 radical addition of either 2,2,5-trimethyl-3-(1-phenylethoxy)-4-phenyl-3-azahexane (TIPNO-based alkoxyamine the "Universal Alkoxyamine", UA) or the SG1-based BlocBuilder (BB) alkoxyamine. Graft polymerizations of styrene and n-butyl acrylate were conducted, using homogeneous media to ensure uniform grafting onto the CTS backbone. The graft modified CTS based materials were analysed by ^{1}H-NMR, TGA and FT-IR.

Introduction

Chitosan (CTS) is a derivative of chitin, which is the second most abundant natural polymer in the world.[1-3] Once chitin has been at least 50% N-deacetylated, it is referred to as CTS. CTS, composed of β (1→4)-links to 2-amino-2-deoxy-D-glucopyranose and to 2-acetamido-2-deoxy-D-glucopyranose, finds applications in water and wastewater treatment, agriculture, biopharmaceuticals, cosmetics and toiletries, and the food and beverages industries.[1-3] It is valued for its biocompatibility, biodegradability, biological tolerance, and resistance against growth bacteria, viruses, and fungi.[1-5] However the properties of native CTS are often not suitable for certain applications, motivating researchers to modify CTS by attaching functional groups or grafting polymeric chains with the objective of obtaining new CTS-based materials with properties tailored for specific applications. The primary limitation in attempts to modify CTS is its insolubility in common organic solvents, being only soluble in acidic aqueous media (pH < 6.5), due to protonation of the primary amino group on the C-2 position of the D-glucosamine repeating unit.[6]

In order to improve CTS solubility in organic solvents, several groups have reported the attachment of functional groups to the CTS backbone chain that allows its solubilization in organic media. Kurita et al.[7] functionalized the amino group of CTS with phthalic anhydride. The resulting N-phthaloylchitosan swelled in pyridine, N,N-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). Dephthaloylation was achieved with hydrazine at 80°C, but later Makuška et al.[8] reported that this process results in the breakdown of the chitosan backbone, and Lebouc et al.[9] found that ~20% of phthaloyl groups remain in the final product. Cai et al.[10, 11] reported the synthesis of sodium dodecylsulfate (SDS)/chitosan complexes (SCC) which are soluble in DMSO. The SDS was successfully removed from the modified complexes by simple precipitation of the SCC DMSO solution in tris(hydroxylmethyl)amino-methane aqueous solution.[10, 11] These N-phthaloylchitosan and SCC have been used as precursors for the synthesis of new CTS-based materials.[10, 11]

Modification of CTS involving graft polymer chains has been mainly achieved via free radical polymerization (FRP),[12-16] ring-opening polymerization (ROP),[14, 17] γ-radiation,[12-14] and cationic polymerization[13, 18] by both *grafting from* and *grafting to* approaches, but there are only a few reports addressing any of the living/controlled radical polymerization (L/CRP) or reversible deactivation radical polymerization (RDRP) techniques or their variations.[19, 20] Atom transfer radical polymerization (ATRP) has been the most used technique for CTS modification.[11, 21-27] CTS modification via reversible addition–fragmentation chain transfer (RAFT)[28-30] and nitroxide-mediated polymerization (NMP)[31-34] has also been reported. With respect to the use of NMP, CTS-TEMPO, obtained by functionalizing N-phthaloylchitosan with 4-OH-TEMPO through γ-radiation, was used as a macroalkoxyamine for the graft polymerization of styrene (ST)[31] and 4-styrenesulfonate (SS).[32] Lefay et al.[33] functionalized the amino group of CTS with acrylamide groups followed by intermolecular 1,2 radical addition of the BlocBuilder alkoxyamine to generate a CTS-SG1 macroalkoxyamine, which enabled the heterogeneous graft polymerization of SS (obtaining a material with a proportion of grafted polymer in close to 30 wt%) or methyl methacrylate (MMA)-acrylonitrile (AN) (getting copolymer with a proportion of graft copolymer close to 20 wt%).[33] Our group recently reported the synthesis of chitosan-graft-poly(styrene-maleic anhydride)-OH-TEMPO.[34] In the first step CTS was functionalized with TEMPO moieties using a previously synthesized Br-OH-TEMPO salt to generate

CTS-TEMPO. Then, the grafting copolymerization of CTS-TEMPO with ST-maleic anhydride (MA) was carried out in supercritical carbon dioxide ($scCO_2$). More recently, we reported modification of CTS by grafting homopolymers and copolymers, previously synthesized via NMP, using a *grafting to* approach.[35] However the *grafting to* approach is desirable for low molecular weight polymers only.[35]

In this work, we report the modification of CTS with polystyrene (PS) and polybutyl acrylate (PBA) via NMP and a *grafting from* approach. To enable a homogeneous graft polymerization, which provides both more uniform grafting and higher grafting efficiency compared to a heterogeneous reaction, we first functionalized CTA with glycidyl methacrylate (GMA) to obtain CTS-*g*-GMA which was then functionalized with SDBS (sodium dodecylbenzenesulfonate) to yield a CTS-SDBS-*g*-GMA complex. The CTS-SDBS-*g*-GMA, was converted into a macroalkoxyamine by the intermolecular 1,2 radical addition of either: (1) the TIPNO-based alkoxyamine, 2,2,5-trimethyl-3-(1-phenylethoxy)-4-phenyl-3-azahexane, commonly referred to as the Universal Alkoxyamine (UA),[36] to yield CTS-SDBS-*g*-GMA-UA; or (2) the SG1-based alkoxyamine, BlocBuilder (BB) to yield CTS-SDBS-*g*-GMA-BB. The CTS-based macroalkoxyamines were then used to initiate the homogeneous graft polymerization of styrene and butyl acrylate in organic media (DMSO) at 115°C. The resulting materials were analysed by ^{1}H-NMR, TGA and FT-IR. To the best of our knowledge, this is the first reported use of TIPNO for graft polymerization from CTS, and the first homogeneous graft polymerization from CTS using SG1.

Experimental

Materials

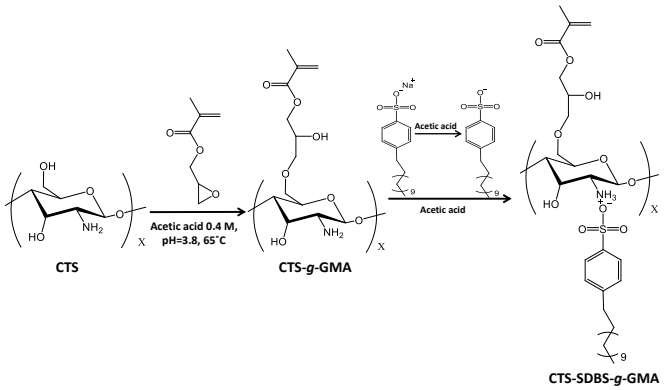
Chitosan (CTS, Aldrich, degree of deacetylation of 85%), glycidyl methacrylate (GMA, Aldrich, 97%), sodium dodecylbenzenesulfonate (SBDS, Aldrich), hydroquinone (Fisher), acetic acid (Fisher, 99.7%), acetonitrile (Fisher, 99.9%), tetrahydrofuran (THF, ACP, 99+%), N,N-dimethylformamide (DMF, Aldrich, 99.8%), methanol (ACP, 99.8%), dimethyl sulfoxide (DMSO, Fisher, 99.9%) deuterium oxide (Cambridge Isotope Laboratories, D 99.9%), dimethyl sulfoxide-d6 (DMSO-d6, Cambridge Isotope Laboratories, D 99.9%), chloroform-d ($CDCl_3$, Aldrich, D 99.8%), TIPNO (2,2,5-Trimethyl-4-phenyl-3-azahexane-3-nitroxide) (T, ≥88 %, Aldrich), Universal Alkoxyamine (UA, Aldrich), Tris(hydroxymethyl)aminomethane (Tris, ≥99.8 %, Aldrich) were used as received. Styrene (St, Aldrich, 99+%) and butyl acrylate (BA, Aldrich, 99+%) were passed over a column containing basic aluminum oxide (Aldrich, ~150 mesh, 58 Å) to remove the inhibitor and stored below 5°C prior to polymerization. SG1 (N-tert-butyl-N-(1-diethylphosphono-2,2-dimethylpropyl) nitroxide) (85%) and BlocBuilder (BB, N-(2-methylpropyl)-N-(1-diethylphosphono-2,2-imethylpropyl)-O-(2-carboxylprop-2-yl) hydroxylamine (BB, 99%) and were kindly supplied by Arkema.

Instrumentation

^{1}H NMR spectroscopy was performed on an FT-NMR Bruker Avance 400 MHz spectrometer with a total of 256 scans, at room temperature using DMSO-d6, $CDCl_3$, or D_2O/CH_3COOH 0.4 M as solvent at 5 mg/mL. Fourier Transform Infrared (FT-IR) spectroscopy was carried out on a Thermo Scientific Nicolet 6700 instrument using an Attenuated Total Reflectance (ATR) accessory equipped with a diamond crystal. A total of 64 scans were co-added per spectrum. Thermogravimetric analysis (TGA) was performed using a TA Instruments Q500 TGA analyser by heating the sample using the following ramp: 10°C min^{-1} from 30 to 75°C, held for 30 min at a plateau of 75°C, and 10 °C min^{-1} to 600°C.

Synthesis of CTS-SDBS-*g*-GMA

CTS was functionalized with GMA and SDBS following previous reports. [10,11,16,35] CTS (1 g) was dissolved in 100 mL 0.4 M acetic acid solution in a three neck round bottom flask, then 5 mL of 0.05 M KOH and a hydroquinone solution ($9.09*10^{-5}$ mol in 10 mL of H_2O) were added to the reaction mixture. Finally, GMA (0.024 mol, 3.53 g, 3.30 mL) was added to the system dropwise. The reaction mixture was previously degassed for 30 minutes under nitrogen atmosphere prior to increasing the temperature to 65°C and magnetically stirred for 2 h. The final pH of the mixture was 3.8. After reaction, CTS-*g*-GMA was precipitated in acetonitrile, filtered, washed three times in clean THF, and dried under vacuum. The dried CTS-*g*-GMA was dissolved in 100 mL of 2% acetic acid solution. SDBS (0.0248 mol, 8.657 g) was also dissolved in 100 mL of 2% acetic acid solution. The SDBS solution was then added dropwise to the CTS-*g*-GMA solution under vigorous magnetic stirring for 2 h. The precipitated CTS-SDBS-*g*-GMA was filtered, washed three times with H_2O and methanol, and dried under vacuum (Scheme 1). CTS-*g*-GMA and CTS-SDBS-*g*-GMA were analysed by ^{1}H NMR, TGA and FT-IR.



Scheme 1. Schematic illustration of the synthesis of CTS-SDBS-*g*-GMA.

The degree of functionalization of CTS with GMA of 11% mol, and 46% mol of CTS-*g*-GMA with SDBS groups. ^{1}H NMR spectra of CTS-*g*-GMA and CTS-SDBS-*g*-GMA are shown in Figure 1. The ^{1}H NMR for CTS-g-GMA (Fig. 1a) shows peaks at 3.09 (2), 3.67 (5-6), 3.83 (3-6), and 4.52 (1) ppm respectively attributed glucosamine ring of CTS. Peaks at 4.24 (7, 8) ppm are attributed to the protons of GMA which are closest to the ether linkage with CTS. Peaks at 5.71 (11) and 6.11(10) ppm are attributed to the vinyl protons of the GMA unit. The spectrum for CTS-SDBS-*g*-GMA (Fig. 1b) shows the signals attributed to the aliphatic chain from SDBS at 0.88 (20), 1.24 (18, 19), & 1.56 (17) ppm. The displacement attributed to the CTS ring appears from 3.00 to 3.60 (3-6) ppm. The signals at 4.14 (7, 8), 5.76 (11), and 6.15 (10) ppm correspond to the -CH_2 and double bond groups from GMA, respectively. At 7.11 and 7.52 ppm the corresponding displacements of the phenyl group from SDBS are observed.

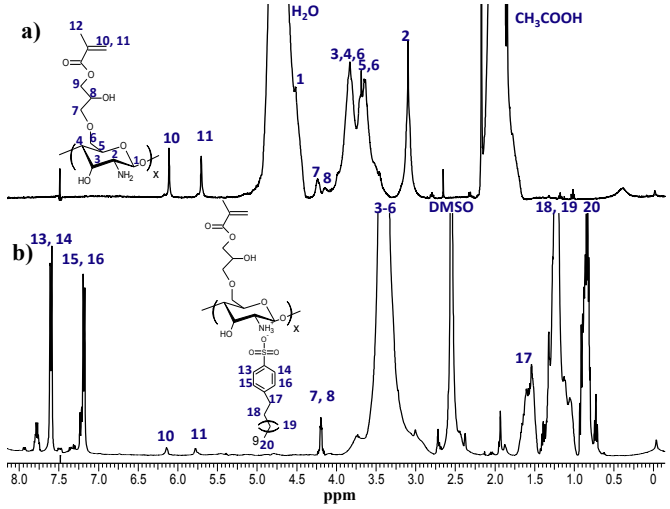
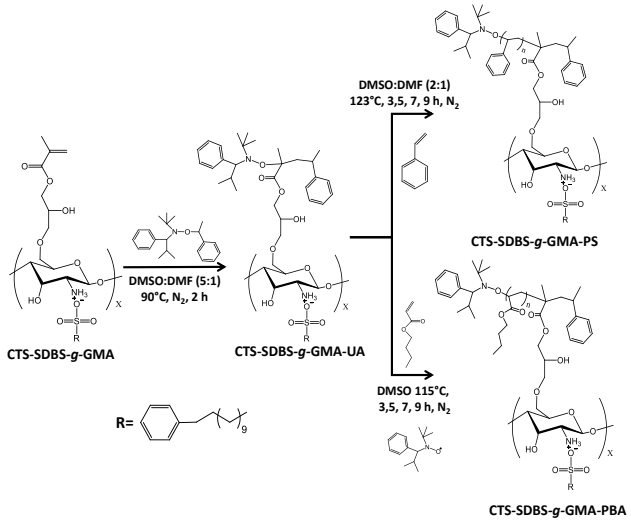


Figure 1. ^{1}H NMR spectra of (a) CTS-*g*-GMA in D_2O/CH_3COOH 0.4 M, and (b) CTS-SDBS-*g*-GMA in DMSO-d6.

Synthesis of CTS-SDBS-*g*-GMA-UA Macroalkoxyamine

In a three neck round bottom flask, 0.5 g of CTS-SDBS-*g*-GMA was solubilized in 100 mL of DMSO, and the Universal Alkoxyamine (0.5044 g, 1.55 mmol) was dissolved in 20 mL of DMF. After complete dissolution, the DMF solution was poured into DMSO solution and then deoxygenated for 30 minutes under nitrogen atmosphere prior to increasing the temperature to 90°C and magnetically stirred for 2 h (Scheme 2). After reaction, the flask was cooled, and the product was precipitated in ethyl acetate, filtered, washed three times with THF, dried under vacuum, and finally analysed by ^{1}H NMR, TGA and FT-IR.



Scheme 2. General procedure of CTS-SDBS-*g*-GMA functionalization with UA and graft polymerization reactions.

The UA was introduced to the CTS-SDBS-*g*-GMA by an intermolecular 1,2 radical addition process, [36] to yield CTS-SDBS-*g*-GMA-UA. ^{1}H NMR spectra of UA and CTS-SDBS-*g*-GMA-UA are shown in Figure 2. The spectrum for CTS-SDBS-*g*-GMA-UA shows, in addition to the characteristic signals of CTS-SDBS-*g*-GMA, new peaks attributed mainly to the UA linked to GMA. From 7.18 to 7.42 (22, 27) ppm are the signals attributed to the phenyl group, at 4.88 (20), 1.99 (25) and 0.5 (26) ppm are the peaks attributed to -CH and -CH_3 groups.

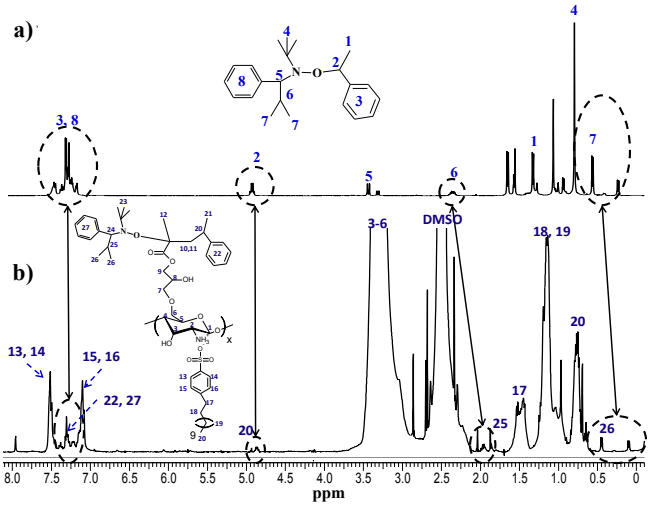
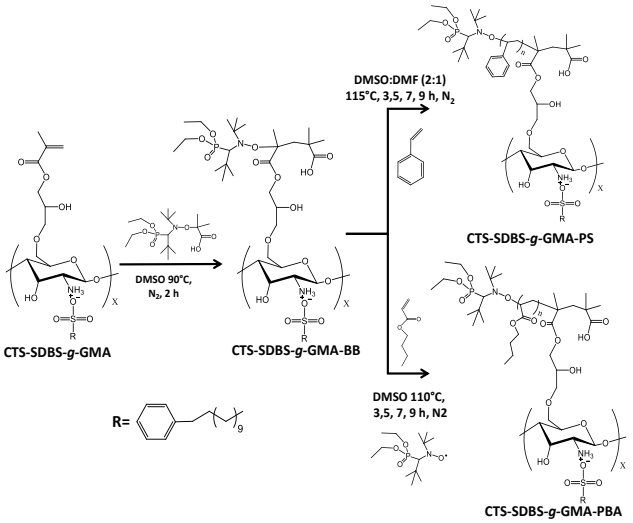


Figure 2. ^{1}H NMR spectra of (a) UA in $CDCl_3$ and (b) CTS-SDBS-*g*-GMA-UA in DMSO-d6.

Synthesis of CTS-SDBS-*g*-GMA-BB Macroalkoxyamine

The synthesis route for CTS-SDBS-*g*-GMA-BB was similar to the CTS-SDBS-*g*-GMA-UA route with slight modifications. CTS-SDBS-*g*-GMA (0.5 g) was solubilized in 100 mL of DMSO. BB (0.5912 g, 1.55 mmol) was also solubilized in 20 mL of DMSO. After complete dissolution, both solutions were mixed, deoxygenated with nitrogen for 30 min prior to increasing the temperature to 90°C under magnetic stirring. The system was kept under these conditions for 2 h (Scheme 3). Following the reaction, CTS-SDBS-*g*-GMA-BB was precipitated in ethyl acetate, filtered, washed three times with clean THF, dried under vacuum, and finally analysed by ^{1}H NMR, TGA and FT-IR.



Scheme 3. General procedure of CTS-SDBS-*g*-GMA functionalization with BB and graft polymerization reactions.

Also BB alkoxyamine was introduced to the CTS-SDBS-*g*-GMA by an intermolecular 1,2 radical addition process.[36] The spectrum of CTS-SDBS-*g*-GMA-BB (Fig. 3) shows the previously discussed peaks of the CTS-SDBS-*g*-GMA and new peaks at 1.18 (23, 25),

1.35 (27) and 1.65 (21, 22) ppm primarily attributed to -CH_3 protons from BB. The other signals attributed to -CH and -CH_2 groups from BB are overlapped with the peaks from the SDBS group. In the spectra of CTS-SDBS-*g*-GMA-UA and CTS-SDBS-*g*-GMA-BB, the peaks at 5.76 and 6.15 ppm assigned to the vinyl group from GMA disappeared, confirming that all of GMA groups were functionalized with alkoxyamine.

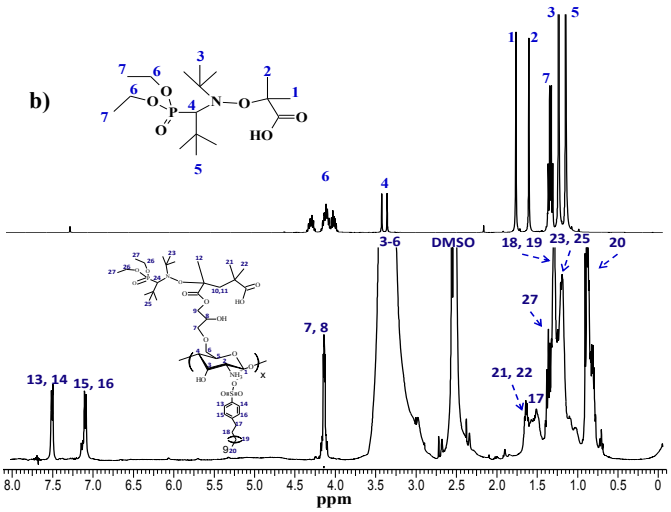


Figure 3. ^{1}H NMR spectra of (a) BB in $CDCl_3$ and (b) CTS-SDBS-*g*-GMA-BB in DMSO-d6.

***Grafting from* Polymerization using TIPNO-based alkoxyamine: CTS-SDBS-*g*-GMA-PS & CTS-SDBS-*g*-GMA-PBA**

St (0.0216 mol, 2.25 g) dissolved in 10 mL of DMF was poured into a DMSO solution of CTS-SDBS-*g*-GMA-UA (0.1 g in 20 mL. The mixture was deoxygenated with nitrogen for 30 min, and stirred for 3, 5, 7 and 9 h at 123°C. CTS-SDBS-*g*-GMA-PS was precipitated in ethyl acetate, filtered, washed at least three times with THF, dried under vacuum and analysed by ^{1}H NMR and TGA. In order to obtain CTS-SDBS-*g*-GMA-PBA, 0.1 g of CTS-SDBS-*g*-GMA and BA (0.0216 mol, 2.77 g) were solubilized in 30 mL of DMSO. TIPNO (0.009 mmol, solubilized in 3 mL of DMF) was added to the mixture. The system was deoxygenated with nitrogen for 30 min, and magnetically stirred for 3, 5, 7, and 9 h at 115°C (see Scheme 2). The products were recovered by precipitation in ethyl acetate, filtered, washed in THF several times, and dried under vacuum prior to analysis by ^{1}H NMR and TGA.

***Grafting from* Polymerization using SG1-based alkoxyamine: CTS-SDBS-*g*-GMA-PS & CTS-SDBS-*g*-GMA-PBA**

0.1 g of CTS-SDBS-*g*-GMA-BB was solubilized in 20 mL of DMSO. St (0.0216 mol, 2.25 g) was dissolved in 10 mL of DMF. Both solutions were mixed, deoxygenated with nitrogen for 30 min, and magnetically stirred for 3, 5, 7, and 9 h at 115°C. CTS-SDBS-*g*-GMA-PS was recovered by precipitation in ethyl acetate, filtered, washed in THF several times, and dried under vacuum prior to analysis by ^{1}H NMR and TGA. For the synthesis of CTS-SDBS-*g*-GMA-PBA, 30 mL of a DMSO solution containing CTS-SDBS-*g*-GMA-BB (0.1 g), BA (0.0216 mol, 2.77 g), and SG1 (0.0009 mmol) was deoxygenated with nitrogen for 30 min, magnetically stirred for 3, 5, 7, and 9 h at 110°C (see Scheme 3). The products were precipitated in ethyl acetate, filtered, washed in THF several times, dried under vacuum, and analysed by ^{1}H NMR and TGA.

Removal of SDBS from graft copolymers

The removal of the SDBS group was performed following previously reported procedures.[10, 11, 35] The modified CTS graft copolymers were dissolved in DMSO (5 mg/mL). The DMSO solution was poured into a Tris solution (pH= 9.0, adjusted with 5 M HCl), stirred magnetically for 2 h at 45°C, and then sonicated for 20 min. The precipitated product was filtered and washed several times with water and methanol to remove free SDBS. The CTS graft copolymers, now without the SDBS group, were analysed by FT-IR.

Results and Discussions

Graft polymerizations of St and BA from CTS functionalized with either TIPNO alkoxyamine (CTS-SDBS-*g*-GMA-UA) or SG1 alkoxyamine (CTS-SDBS-*g*-GMA-BB) were conducted in DMSO. SG1 and TIPNO are versatile nitroxides capable of mediating the polymerization of acrylic esters, vinyl pyridines, acrylonitrile, acrylamides, as well as styrene and its derivatives.[37, 38] SG1 has been widely used to modify different substrates via a *grafting from* approach such as nanoparticles,[36, 39] microspheres,[40] and CTS.[33] TIPNO has been used to modify substrates (with graft polymers) such as silicon wafers[41] or γFe_2O_3 nanoparticles[42] but it has not been used to produce graft polymers on CTS or any biopolymer.

***Grafting from* polymerizations:**

1. CTS-SDBS-*g*-GMA-PS and CTS-SDBS-*g*-GMA-PS via TIPNO mediated polymerization

Grafting from polymerizations of St and BA, at 123°C and 115°C respectively, were conducted using DMSO as solvent for reaction times of 3, 5, 7, and 9 h. In the case of the St grafting reaction, 50% of DMF with respect to the total of DMSO used was also used in order to maintain the homogeneity given the insolubility of PS in DMSO. The CTS-SDBS-*g*-GMA-PBA and CTS-SDBS-*g*-GMA-PS graft products were washed at least three times with THF, as the products were soluble in DMSO and insoluble in any other organic solvent, to remove ungrafted polymer chains. The fraction of monomer converted into graft polymer was calculated by gravimetry, and the composition of the resulting materials was determined (Table 1). Assuming 100% initiation efficiency, the M_n of the graft chains for BA and St polymerizations were estimated (Table 1). This assumption likely result in underestimation of the actual M_n but it is not possible to accurately determine the actual initiation efficiency.

Table 1. Monomer graft conversion (MGC), percentage of grafted polymer determined by gravimetry and TGA, and theoretical M_n of St and BA grafting reactions from CTS-SDBS-*g*-GMA-UA at various reaction times.

Grafting reaction	Time (h)	MGC (%) (± σ)	Graft polymer (%)[a]	Graft Polymer (%)[b]	Mn,th (Da)
St	3	1.44 (±0.04%)	26	26	870
	5	2.88 (±0.08%)	42	46	1090
	7	4.80 (±0.05%)	55	53	1820
	9	7.20 (±0.11%)	65	63	2730
BA	3	1.15 (±0.06%)	24	22	840
	5	1.58 (±0.09%)	30	28	1030
	7	2.02 (±0.05%)	36	32	1220
	9	2.92 (±0.08%)	45	40	1620

[a]Percentage of graft polymer on CTS-SDBS-*g*-GMA determined by gravimetry.
[b]Percentage of graft polymer on CTS-SDBS-*g*-GMA determined by TGA.

As is shown in Figure 4, BA was consumed approximately linearly as the reaction proceeded while for St slight upward curvature was

observed. The slower consumption of BA in comparison of St is attributed to the presence of the deliberately added free TIPNO (approximately 5% of excess with respect to the total of TIPNO linked to CTS) which suppresses the polymerization rate. With styrene, thermal initiation generates additional radicals that consume excess nitroxide and alleviates this problem. The addition of free nitroxide in acrylate polymerization reactions and a low reaction temperature decrease the rate of polymerization and therefore afford better control.[43] For both grafting reactions, a large excess of monomer was used in order to promote the grafting reaction. The final monomer conversion ranged from 1.4 to 7% for PS and 1.15 to 2.92% for PBA in the range from 3 to 9 h; however, this was enough to achieve relatively high degrees of grafting.

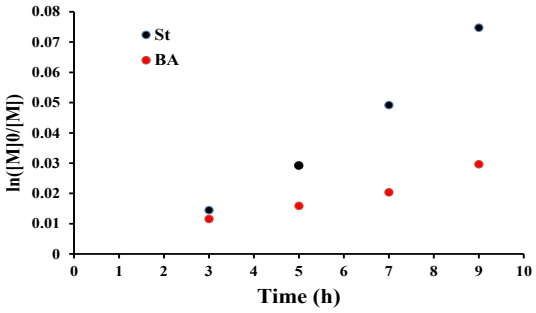


Figure 4. Evolution of the monomer converted into graft polymer (MGC) for the polymerization of BA and St from CTS-SDBS-*g*-GMA-UA at 115 and 123°C respectively for 3, 5, 7, and 9 h.

TGA measurements for the CTS-SDBS-*g*-GMA-PS (obtained via TIPNO-mediated polymerization) at 3, 5, 7, and 9 h (Fig. 5) reveal an initial weight loss from ~200 to 250°C due to SDBS decomposition. From ~275 to 400°C, a continuous loss and a change in the slope were noted, attributed to the degradation of both CTS and PS chains. Assuming that all CTS-SDBS-*g*-GMA is decomposed by 350°C and PS decomposes after 350°C, the percentage of PS grafted to the CTS backbone chain was estimated. The PS grafted from CTS-SDBS-*g*-GMA was 26, 46, 53 and 63% of the total graft polymer mass at 3, 5, 7, and 9 h respectively which is in good agreement with the values determined by gravimetry (Table 1).

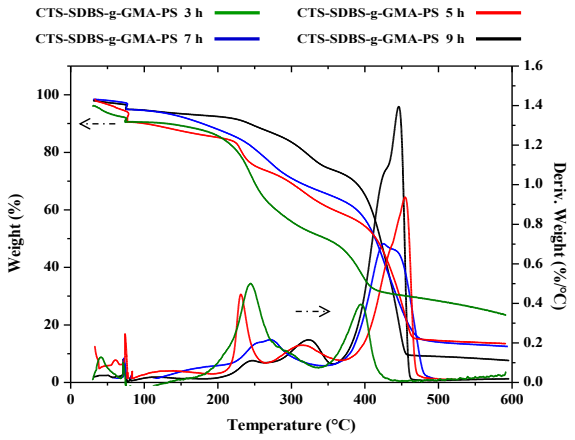


Figure 5. TGA of CTS-SDBS-*g*-GMA-PS obtained at 3, 5, 7, and 9 h via TIPNO mediated polymerization.

The ^{1}H NMR spectra of CTS-SDBS-*g*-GMA-PS obtained at different reaction times via TIPNO mediated polymerization (Fig. 6) indicate signals for the SDBS groups at 0.88 (20), 1.24 (18, 19), 1.56 (17), 7.11 (15, 16), and 7.52 (13, 14) ppm. The corresponding signals attributed to the phenyl group from PS appear at 7.10 (overlapped with the signal of SDBS) and 6.58 (23-27) ppm.

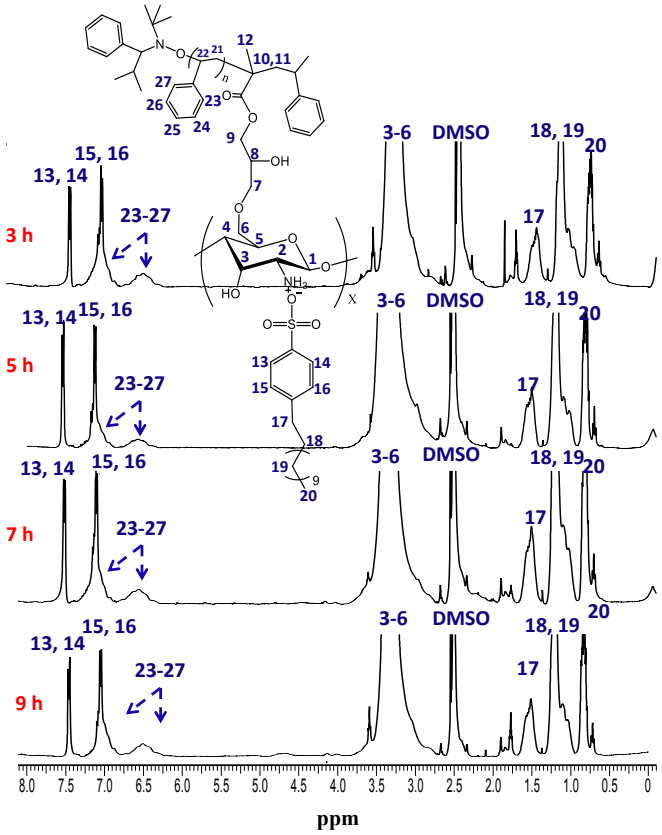


Figure 6. ^{1}H NMR spectrum in DMSO-d6 of CTS-SDBS-*g*-GMA-PS obtained via TIPNO mediated polymerization at 3, 5, 7, and 9 h.

Thermograms for CTS-SDBS-*g*-GMA-PBA (obtained via TIPNO-mediated polymerization) at 3, 5, 7, and 9 h, shown in Fig. 7, support the presence of PBA grafted to CTS-SDBS-*g*-GMA in different proportions, as a function of reaction time. The decomposition of SDBS is observed from ~200 to 250°C, and from ~250 to 400°C the degradation of both CTS and PBA chains is noted. Based on the same assumptions made for the St grafting reactions, the percentage of PBA grafted CTS-SDBS-*g*-GMA-UA determined by TGA was 22, 28, 32, and 40% for 3, 5, 7, 9 h respectively.

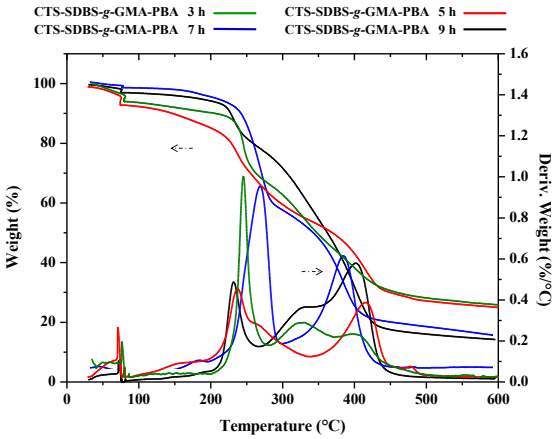


Figure 7. TGA of CTS-SDBS-*g*-GMA-PBA obtained at 3, 5, 7, and 9 h via TIPNO mediated polymerization.

Figure 8 shows the ^{1}H NMR spectra of CTS-SDBS-*g*-GMA-PBA (obtained via TIPNO-mediated polymerization) at 3, 5, 7, and 9 h. The spectra show new peaks at 1.29 ppm and 4.01 (23) ppm, attributed to the butyl ester of PBA, and the corresponding signals of SDBS groups at 0.88 (20), 1.24 (18, 19), 1.56 (17), 7.11 (15, 16), and 7.52 (13, 14) ppm.

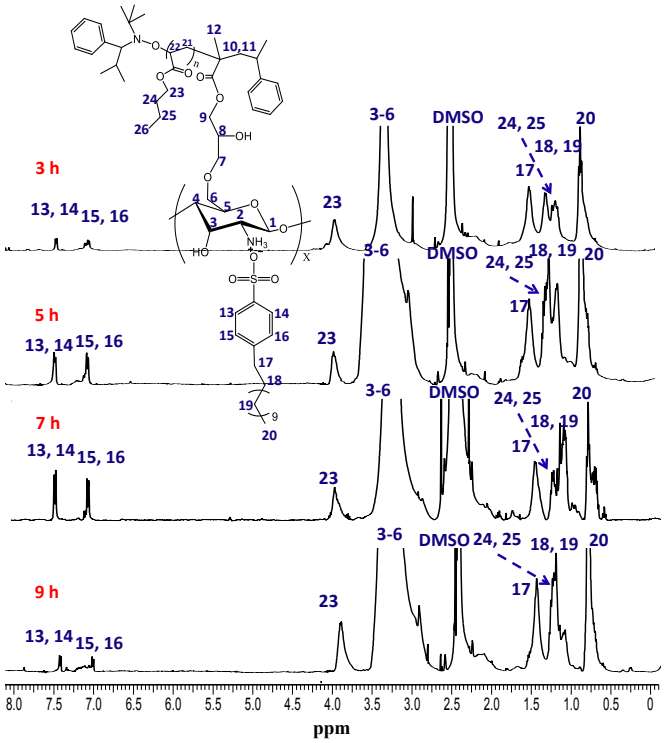


Figure 8. ^{1}H NMR spectrum in DMSO-d6 of CTS-SDBS-*g*-GMA-PBA obtained via TIPNO mediated polymerization at 3, 5, 7, and 9 h.

Grafting from polymerizations: 2. CTS-SDBS-*g*-GMA-PS and CTS-SDBS-*g*-GMA-PS via SG1 mediated polymerization

The SG1 macroalkoxyamine, CTS-SDBS-*g*-GMA-BB, was used to carry out grafting reactions of St and BA at 115 and 110°C respectively, for 3, 5, 7, and 9 h. In the case of St, and as shown in Fig. 9, in each reaction the product precipitated out of the continuous phase as the graft polymer grew. This phenomenon may be attributed to crosslinking reactions between the growing graft chains that occur when they are active, which is favoured by the presence of a polar solvent like DMSO. Polar solvents produce a stable polar resonance structure resulting in slower deactivation of the propagating radicals and therefore a faster reaction rate and a greater propensity for mutual termination between chains on different CTS molecules. [44, 45] Crosslinking arising from coupling between the growing graft chains were not observed when CTS-SDBS-*g*-GMA-UA was used to carry out *grafting from* reactions mainly attributed to a slower rate of polymerization. The differences between TIPNO- and SG1-mediated polymerization rates can also be attributed to the polarity on the reaction media since in the TIPNO-mediated polymerization a less polar medium is used (mixture of DMSO:DMF) compared to SG1-mediated polymerization (DMSO). In the case of the BA grafting reactions, homogeneity was maintained in each reaction, as shown in Fig. 9. CTS-SDBS-*g*-GMA-PBA was insoluble in THF, DMF, 1-4 dioxane, water, and acetic acid solutions, and soluble only in DMSO. To ensure that the product was not mixed with PBA homopolymer, it was washed at least three times with clean THF after precipitation. The percentage of monomer converted to graft polymer, composition of the resulting graft polymers and the theoretical molecular weight M_n (from the SG1 molar concentration present in the mixture) are shown in Table 2.

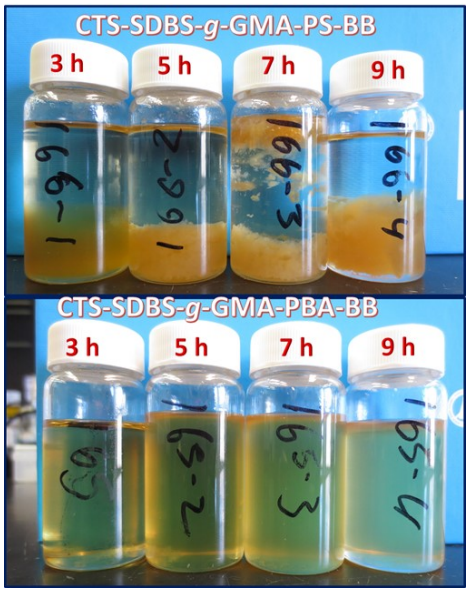


Figure 9. CTS-SDBS-*g*-GMA-PS and CTS-SDBS-*g*-GMA-PBA obtained via SG1-mediated polymerization at various reaction times.

Table 2. Monomer graft conversion (MGC), composition, and theoretical M_n of BA grafting reactions from CTS-SDBS-*g*-GMA-BB at predetermined reaction times.

Grafting reaction	Time (h)	MGC (%) (± σ)	Graft polymer (%)[a]	Graft Polymer (%)[b]	Mn,th (Da)
BA	3	0.68 (±0.02%)	16	14	627
	5	1.01 (±0.06%)	22	19	774
	7	3.06 (±0.05%)	46	42	1689
	9	4.33 (±0.04%)	55	52	2250

[a]Percentage of graft polymer on CTS-SDBS-*g*-GMA determined by gravimetry.
[b]Percentage of graft polymer on CTS-SDBS-*g*-GMA determined by TGA.

In the SG1-mediated BA grafting reaction, free SG1 (approximately 5% of excess with respect to the total of SG1 linked to CTS) was added to the mixture in order to decrease the rate of polymerization, avoid possible termination reactions and therefore enhance the control over the polymerization. AS it is shown in Fig. 10 BA was consumed approximately linearly as the reaction proceeded and indicates an increment in the rate of polymerization after the fifth hour, possibly due to consumption of the free SG1. From 3 to 9 h, the monomer conversion ranged from 0.68 to 4.33% (Table 2).

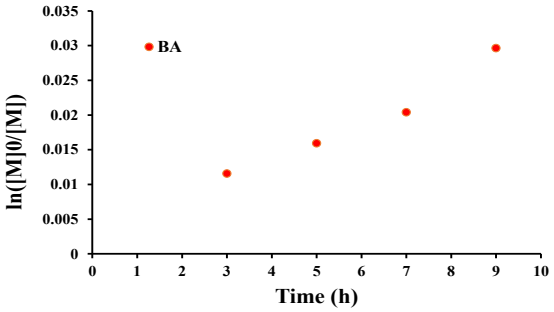


Figure 10. Evolution of the monomer converted into graft polymer (MGC) for the polymerization of BA from CTS-SDBS-*g*-GMA-BB at 115° C for 3, 5, 7, and 9 h.

CTS-SDBS-*g*-GMA-PBA obtained at 3, 5, 7, and 9 h was analysed by TGA (Fig. 11). The thermograms indicate the loss attributed to SDBS groups (~200-250°C), and from ~250 to >400°C to the decomposition of CTS and PBA chains. TGA results confirmed very similar values to those obtained by gravimetric analyses (Table 2) for the amount of PBA grafted to CTS-SDBS-*g*-GMA-BB (14, 19, 42 and 52%, assuming that all CTS-SDBS-*g*-GMA decomposes by 350°C) at 3, 5, 7, and 9 h, respectively.

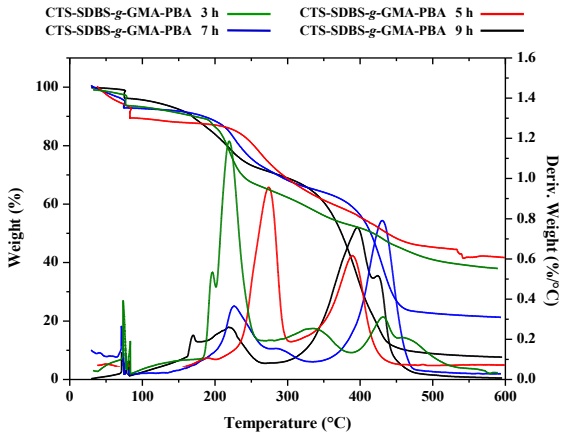


Figure 11. TGA of CTS-SDBS-*g*-GMA-PBA obtained at 3, 5, 7, and 9 h via SG1 mediated polymerization via SG1-mediated polymerization.

In Figure 12, ^{1}H NMR spectra of CTS-SDBS-*g*-GMA-PBA at 3, 5, 7, and 9 h show new peaks attributed to the butyl ester of PBA at 1.29 (24, 25) ppm and 4.01 (23) ppm.

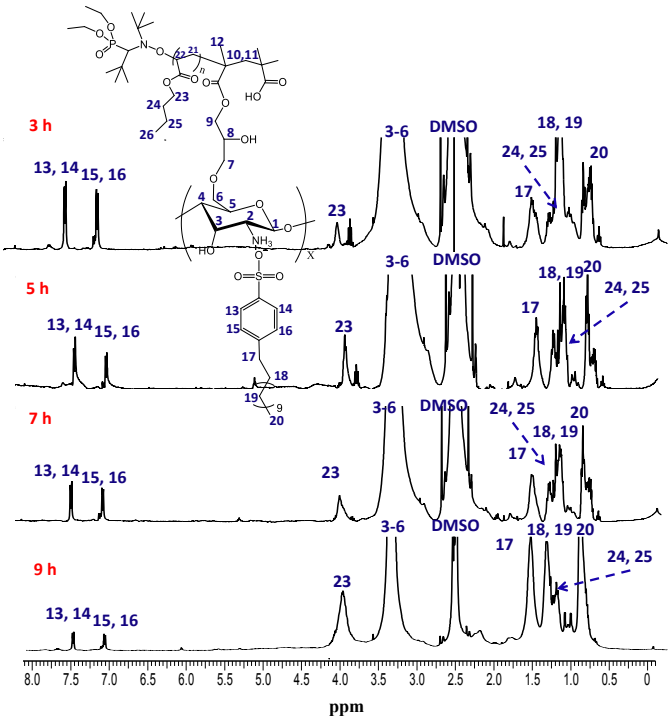


Figure 12. ^{1}H NMR spectrum in DMSO-d6 of CTS-SDBS-*g*-GMA-PBA obtained via SG1-mediated polymerization at 3, 5, 7, and 9 h.

Removal of SDBS from graft copolymers

SDBS was successfully removed from CTS-SDBS-*g*-GMA-PS and CTS-SDBS-*g*-GMA-PBA obtained via TIPNO- and SG1-mediated polymerization, to yield CTS-*g*-GMA-PS and CTS-*g*-GMA-PBA. The resulting materials were insoluble in THF, DMF, 1-4 dioxane, DMSO, water, acetic acid solutions or their mixtures.

Figure 13 shows the FT-IR spectra for CTS, CTS-SDBS-*g*-GMA, CTS-*g*-GMA-PS and CTS-*g*-GMA-PBA, both obtained via TIPNO-mediated polymerization, and CTS-SDBS-*g*-GMA-PBA obtained via SG1-mediated polymerization. The CTS spectrum (Fig. 13a) shows several peaks attributed to its functional groups. A strong band at 3448 cm^{-1} from the stretching vibration of the O-H bonds; stretching vibrations from C-H (υ, C-H) at 2875 cm^{-1}; stretching vibrations of C-O bonds (νs, C-O) of the remaining amide group at 1652 and 1606 cm^{-1}; bending from –CH_2 groups at 1414 cm^{-1}; symmetrical deformation of -CH_3 groups at 1375 cm^{-1}; stretching vibrations of C-O (ν, C-O) bonds between 1160 and 1150 cm^{-1} and at 1088 cm^{-1} the peak from the C-O-C (ν, C-O-C) bond. The spectrum of CTS-SDBS-*g*-GMA (Fig. 13b) shows the contribution of the SDBS group; at 1037 cm^{-1} attributed to S=O, and at 669 cm^{-1}, corresponding to the C-S bond. In the CTS-*g*-GMA-PS (via TIPNO-mediated polymerization) spectrum (Fig. 13c) are noted: aromatic vibrations from the C-H (υs, C-H) in the range 3100–3000 cm^{-1}, vibrations from C-H (υ, C-H) from 2980–2850 cm^{-1}, aromatic overtones at 1954-1800 cm-1, stretching vibrations of C=C bonds (νs, C=C) from the aromatic ring at 1601, 1494, 1451 cm^{-1} and out of the plane vibrations at 704 and 760 cm^{-1}. The spectra of CTS-*g*-GMA-PBA and CTS-*g*-GMA-PBA, obtained via TIPNO- and SG1-mediated polymerization respectively, (Figures 13d and 13e) show the same variety of signals since both materials possess the same functional groups from the CTS and PBA units. The new contributions from PBA that

appear at 2964 cm^{-1} are attributed to C-H from the aliphatic chain and at 1735 cm^{-1} to the stretching vibration of the carbonyl group (vs, C=O). The absence of signals at 1037 and 669 cm^{-1} in Figures 13c, 13d, and 13e indicate that the removal of SDBS was complete.

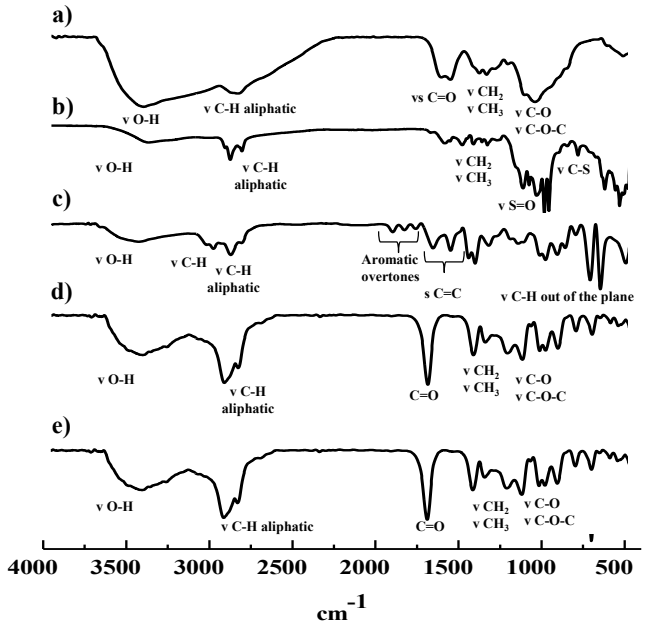


Figure 13. FT-IR of (a) CTS, (b) CTS-SDBS-*g*-GMA, (c) CTS-*g*-GMA-PS and (d) CTS-*g*-GMA-PBA obtained via TIPNO mediated polymerization, and (e) CTS-SDBS-*g*-GMA-PBA obtained via SG1 mediated polymerization.

Conclusions

Graft polymerizations of St and BA on CTS were carried out by NMP in homogeneous media using macroalkoxyamines based on either TIPNO-functionalized or SG1-functionalized CTS. CTS-SDBS-*g*-GMA-UA and CTS-SDBS-*g*-GMA-BB are capable of initiating and mediating the polymerizations and may be considered as potential precursors of new bio-hybrid materials. TIPNO-mediated polymerizations yielded DMSO-soluble graft copolymers with both St and BA, while for SG1-mediated polymerizations only CTS grafted with PBA was soluble in DMSO. The insolubility of the CTS grafted with PS may be attributable to termination of the growing chains leading to crosslinking. The procedures used in this study demonstrate the feasibility of CTS graft modification via NMP using a *grafting from* approach in homogeneous media. Working in homogeneous rather than heterogeneous media, we were able to increase the polymer graft content in CTS at shorter reaction times compared to previous reports.[33] The grafting polymer content can be manipulated by changing the reaction time. NMP allows tailoring of the molecular weight and architecture of the graft chains, and therefore the design and control of final product properties for CTS-based hybrids. One of the principal disadvantages of CTS is its poor mechanical properties (which can be a problem for biomedical applications); the grafted PS and PBA could significantly enhance these properties. Additionally, the approach developed in this study targets OH groups on the CTS molecule, thereby preserving the amino functionality which is fundamentally important as a number of the valuable CTS bioproperties are attributed to the presence of the amino group. For example, the interaction between positively charged chitosan and a negatively charged DNA has also been exploited for delivery of plasmid DNA.[46,47] The -NH_2 groups on the chain are involved in specific interactions with metals so this biopolymer is also widely used for the recovery of heavy metals from various waste waters.[48]

Acknowledgements

The Natural Sciences and Engineering Research Council of Canada (NSERC), the Ontario Research Chairs Program (MFC) and the Canada Research Chairs Program (PC) provided financial support. O. G-V acknowledges CONACyT for his scholarship for Ph.D. studies (224318), and the scholarship (276081) for his studies in Canada.

Notes and references

[a] Department of Chemical Engineering, Queen's University, 19 Division Street, Kingston, Ontario, Canada K7L 3N6.
[b] Polymerization Processes Department. Centro de Investigación en Química Aplicada (CIQA) Blvd. Enrique Reyna No. 140, Saltillo, Coahuila. 25294, México.
[c] Department of Civil Engineering, Queen's University, 58 University Avenue, Kingston, Ontario, Canada K7M 9H7.
See DOI: 10.1039/b000000x/

1. M. N. V. Ravi Kumar, *React. Funct. Polym.*, 2000, **46**, 1-27.
2. M. Rinaudo, *Prog. Polym. Sci.*, 2006, **31**, 603-632.
3. M. Rinaudo, *Polym. Int.*, 2008, **57**, 397-430.
4. H. Sashiwa and S.-i. Aiba, *Prog. Polym. Sci.*, 2004, **29**, 887-908.
5. H. Yi, L.-Q. Wu, W. E. Bentley, R. Ghodssi, G. W. Rubloff, J. N. Culver and G. F. Payne, *Biomacromolecules*, 2005, **6**, 2881-2894.
6. M. Rinaudo, G. Pavlov and J. Desbrières, *Polymer*, 1999, **40**, 7029-7032.
7. K. Kurita, H. Ikeda, Y. Yoshida, M. Shimojoh and M. Harata, *Biomacromolecules*, 2002, **3**, 1-4.
8. R. Makuška and N. Gorochovceva, *Carbohydr. Polym.*, 2006, **64**, 319-327.
9. F. Lebouc, I. Dez, J. Desbrières, L. Picton and P.-J. Madec, *Polymer*, 2005, **46**, 639-651.
10. G. Cai, H. Jiang, K. Tu, L. Wang and K. Zhu, *Macromol. Biosci.*, 2009, **9**, 256-261.
11. C. Kang, L. Yu, G. Cai, L. Wang and H. Jiang, *J. Polym. Sci., Part A: Polym. Chem.*, 2011, **49**, 3595-3603.
12. V. K. Mourya and N. N. Inamdar, *React. Funct. Polym.*, 2008, **68**, 1013-1051.
13. R. Jayakumar, M. Prabaharan, R. L. Reis and J. F. Mano, *Carbohydr. Polym.*, 2005, **62**, 142-158.
14. D. W. Jenkins and S. M. Hudson, *Chem. Rev.*, 2001, **101**, 3245-3274.
15. E. A. Elizalde-Peña, N. Flores-Ramirez, G. Luna-Barcenas, S. R. Vásquez-García, G. Arámbula-Villa, B. García-Gaitán, J. G. Rutiaga-Quiñones and J. González-Hernández, *Eur. Polym. J.*, 2007, **43**, 3963-3969.
16. N. Flores-Ramírez, E. A. Elizalde-Peña, S. R. Vásquez-García, J. González-Hernández, A. Martinez-Ruvalcaba, I. C. Sanchez,

G. Luna-Bárcenas and R. B. Gupta, *J. Biomater. Sci., Polym. Ed.*, 2005, **16**, 473-488.
17. L. Liu, Y. Li, H. Liu and Y. e. Fang, *Eur. Polym. J.*, 2004, **40**, 2739-2744.
18. S. Yoshikawa, T. Takayama and N. Tsubokawa, *J. Appl. Polym. Sci.*, 1998, **68**, 1883-1889.
19. M. Tizzotti, A. Charlot, E. Fleury, M. Stenzel and J. Bernard, *Macromol. Rapid Commun.*, 2010, **31**, 1751-1772.
20. K. Zhang, P. Zhuang, Z. Wang, Y. Li, Z. Jiang, Q. Hu, M. Liu and Q. Zhao, *Carbohydr. Polym.*, 2012, **90**, 1515-1521.
21. H. Bao, J. Hu, L. H. Gan and L. Li, *Journal of Polymer Science Part A: Polymer Chemistry*, 2009, **47**, 6682-6692.
22. K. El Tahlawy and S. M. Hudson, *J. Appl. Polym. Sci.*, 2003, **89**, 901-912.
23. T. Fujie, J. Y. Park, A. Murata, N. C. Estillore, M. C. R. Tria, S. Takeoka and R. C. Advincula, *ACS Appl. Mater. Inter.*, 2009, **1**, 1404-1413.
24. N. Li, R. Bai and C. Liu, *Langmuir*, 2005, **21**, 11780-11787.
25. J. Lindqvist and E. Malmström, *J. Appl. Polym. Sci.*, 2006, **100**, 4155-4162.
26. P. Liu and Z. Su, *Mater. Lett.*, 2006, **60**, 1137-1139.
27. N. H. Munro, L. R. Hanton, S. C. Moratti and B. H. Robinson, *Carbohydrate Polymers*, 2009, **77**, 496-505.
28. D. Hua, J. Tang, J. Cheng, W. Deng and X. Zhu, *Carbohydr. Polym.*, 2008, **73**, 98-104.
29. J. Jiang, X. Pan, J. Cao, J. Jiang, D. Hua and X. Zhu, *Int. J. Biol. Macromol.*, 2012, **50**, 586-590.
30. J. Tang, D. Hua, J. Cheng, J. Jiang and X. Zhu, *Int. J. Biol. Macromol.*, 2008, **43**, 383-389.
31. D. Hua, W. Deng, J. Tang, J. Cheng and X. Zhu, *Int. J. Biol. Macromol.*, 2008, **43**, 43-47.
32. J. Jiang, D. Hua, J. Jiang, J. Tang and X. Zhu, *Carbohydr. Polym.*, 2010, **81**, 358-364.
33. C. Lefay, Y. Guillaneuf, G. Moreira, J. J. Thevarajah, P. Castignolles, F. Ziarelli, E. Bloch, M. Major, L. Charles and M. Gaborieau, *Polym. Chem.*, 2013, **4**, 322-328.
34. O. García-Valdez, D. G. Ramírez-Wong, E. Saldívar-Guerra and G. Luna-Bárcenas, *Macromol. Chem. Phys.*, 2013, **214**, 1396-1404.
35. O. García-Valdez, S. George, R. Champagne-Hartley, E. Saldívar-Guerra, P. Champagne and M. F. Cunningham, *Carbohydr. Polym.* 2014, **CARBPOL-D-14-02804**.
36. D. Gigmes, P.-E. Dufils, D. Gle, D. Bertin, C. Lefay and Y. Guillaneuf, *Polym. Chem.*, 2011, **2**, 1624-1631.
37. R. B. Grubbs, *Polymer Reviews*, 2011, **51**, 104-137.
38. J. Nicolas, Y. Guillaneuf, C. Lefay, D. Bertin, D. Gigmes and B. Charleux, *Prog. Polym. Sci.*, 2013, **38**, 63-235.
39. R. Inoubli, S. Dagréou, M.-H. Delville, A. Lapp, J. Peyrelasse and L. Billon, *Soft Matter*, 2007, **3**, 1014-1024.
40. K. Bian and M. F. Cunningham, *Polymer*, 2006, **47**, 5744-5753.
41. R. V. Ostaci, C. Celle, G. Seytre, E. Beyou, J. P. Chapel and E. Drockenmuller, *J. Polym. Sci., Part A: Polym. Chem.*, 2008, **46**, 3367-3374.
42. W. H. Binder, D. Gloger, H. Weinstabl, G. Allmaier and E. Pittenauer, *Macromolecules*, 2007, **40**, 3097-3107.
43. D. Benoit, V. Chaplinski, R. Braslau and C. J. Hawker, *J. Amer. Chem. Soc.*, 1999, **121**, 3904-3920.
44. S. Marque, H. Fischer, E. Baier and A. Studer, *J. Org. Chem.*, 2001, **66**, 1146-1156.
45. G. Moad and E. Rizzardo, *Macromolecules*, 1995, **28**, 8722-8728.
46. M. Iqbal, W. Lin, I. Jabbal-Gill, S. S. Davis, and M. W. Steward, *Illum L.Vaccine* 2003, **21**, 1478-1485.
47. K. Roy, H-Q. Mao, S-K. Huang, and K. W. Leong. *Nat Med* 1999, **5**, 387-391.
48. R.A.A. Muzzarelli and E.R., Pariser, eds. 1978. Proc. 1st Int. Conf. Chitin/Chitosan Cambridge, MA: MIT Press.