Synthesis of well-defined Locust Bean Gum-graft-copolymers using ambient aqueous atom transfer radical polymerisation†

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The first atom transfer radical graft copolymerisation at ambient temperature in water from a soluble polysaccharide is demonstrated for a range of monomer types.

Polysaccharides, including cellulose, chitin, chitosan, guar gum and dextran, represent the most abundant class of industrial raw materials and have been the subject of much research attention due to their sustainability, biodegradability and scale of production.[1] Modification of these natural materials by graft copolymerisation using vinyl monomers offers the opportunity to tailor their physical and chemical properties, yielding functional macromolecules that may find a wide range of applications.[2]

Several modification techniques have been reported, involving both 'grafting-from' (growth of polymer chains from initiating sites on the polysaccharide backbone) and 'grafting-to' methods (coupling of preformed polymer chains to the polysaccharide).[2] 'Grafting-from' is the most common procedure with initiating sites generated by various chemical methods or irradiation.[2] Currently reported 'conventional' methods of 'grafting-to' polysaccharides give poorly defined materials with respect to control of the number, placement, molecular weight and composition of the grafts.[2]

Controlled polymerisation techniques such as atom transfer radical polymerisation (ATRP)[3] and reversible addition fragmentation chain transfer (RAFT)[4] provide opportunities to control the 'grafting-from' procedure under pseudo-'living' polymerisation conditions. ATRP is a predominantly solvent based technique that often requires high temperatures and long reaction times to achieve significant monomer conversion. Armes and coworkers have reported aqueous-ATRP[5] allowing the rapid, controlled polymerisation at ambient temperatures of water-soluble monomers in protic media, including bulk water and water–alcohol mixtures. Recently in our studies, using isopropanol (IPA)–water mixtures, we have rapidly polymerised the hydrophobic monomer *n*-butyl methacrylate at ambient temperature to high conversion and low polydispersity with excellent control.[6]

Polysaccharides are excellent candidate scaffolds for multifunctional ATRP initiator synthesis as shown by recent reports of the formation of multi-armed star-polymers using simple sugars (for example, glucose; β-cyclodextrin) and a variety of vinyl monomers.[7] ATRP from insoluble polysaccharide-based solid supports such as filter paper,[8] cellulose fibres[9] and cross-linked dextran particles[10] has been reported in the last five years. Heterogeneous ATRP has also been used to graft methoxy capped oligoethyleneoxide methacrylate onto chitosan.[11] Until recently, the use of soluble polysaccharide macroinitiators had only been reported in non-aqueous ATRP using high boiling, polar organic solvents, such as DMF or dioxane,[12,13] although DMF–water systems have very recently been reported by Bontempo *et al.*[14] In our recent patents[15] and current report, the first water-soluble polysaccharide macroinitiators based on Locust Bean Gum (LBG) have been synthesised. We have controlled the number of initiating sites and successfully graft copolymerised a range of monomers at ambient temperature in water.

Locust Bean Gum, **1**, Scheme 1, is a commercially available water-soluble β-1,4-polysaccharide obtained from the seed of the carob tree (*Ceretonia Siliqua L.*). LBG, which is also soluble in LiCl–DMSO solutions, is a galactomannan consisting of a mannose backbone with single side chain galactose units, and typical molar mass quoted in the literature is between 300–360 kg mol^{-1}.

The formation of ATRP initiator sites is a well described process using a range of different approaches.[3,5] In many cases, the reaction of hydroxyl groups with 2-bromoisobutyric acid, **2**, (BIBA) or its acid bromide will produce a tertiary bromide capable of initiation in the presence of a suitable catalyst. Reaction onto LBG to form esters, however, is hampered by the large amount of residual bound water within the polysaccharide (up to 13% w/w) causing rapid hydrolysis of the activated acid. In recent years, we have experimented widely using 1,1′-carbonyl diimidazole (CDI), **3**, as a coupling agent to form a range of new materials including dendrimers and hyperbranched polymers.[16] When reacted with a carboxylic acid, CDI forms an acid imidazolide, which, although reactive towards water, can be reacted with hydroxyl groups without purification and is relatively stable to hydrolysis, depending on the structure of the acid used.[17] In our synthetic approach, a clear homogeneous solution of LiCl–DMSO (6% w/v) was produced by stirring LiCl in anhydrous DMSO at 150 °C. LiCl is important for breaking the hydrogen bonding within the polysaccharide. LBG was dissolved into this solution and subsequently cooled to 65 °C. In separate flasks, **2** and **3** were independently dissolved in anhydrous DMSO. The CDI solution was added dropwise at ambient temperature to **2** to form the acid imidazolide **4**. Once the reaction was complete, **4** was added to the LBG solution and stirred at 65 °C for 24 hours. Precipitation into methanol and subsequent methanol washings and soxhlet extractions yielded the off-white water-soluble macroinitiator, **5**, Scheme 1.

Formation of **5** was readily confirmed by infrared spectroscopy (IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy in D_2O, with the appearance of an ester carbonyl stretch at

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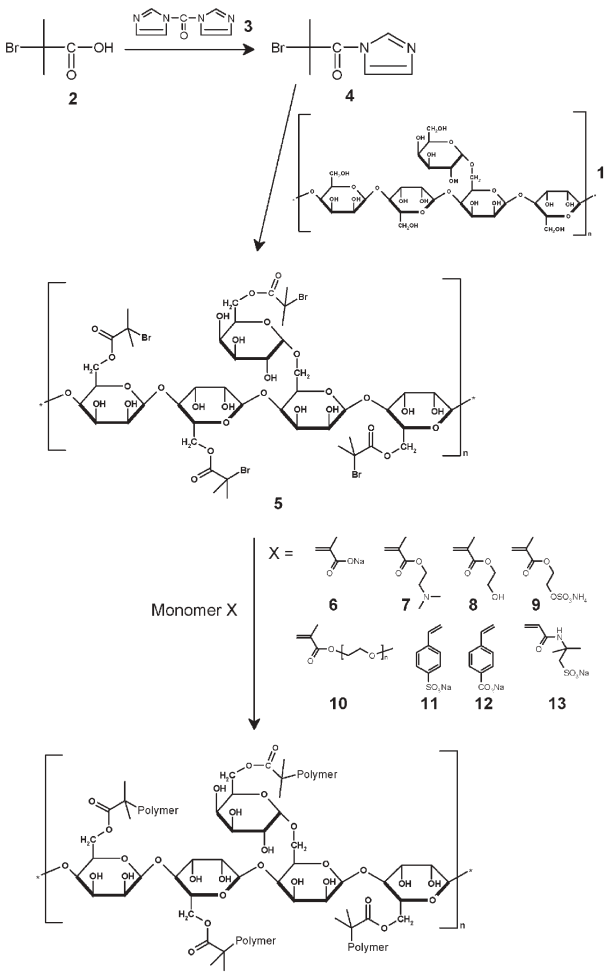

Scheme 1

ν_{max}/cm^{-1} = 1736 (ester CO) and the characteristic tertiary methyl groups of the 2-bromoisobutyrate ester at δ = 1.8 ppm. Degradation of the macroinitiator for 1 hour at 70 °C in a 20% DCl–D_2O mixture was also accomplished. A sharper series of NMR resonances were observed for the sugar protons with multiplets at δ = 3.4–4.2 ppm and δ = 4.6–5.4 ppm, corresponding to the ring and anomeric protons, respectively. The signal at δ = 1.8 ppm was still present after this treatment and allowed for assessment of the extent of ester formation under different reaction conditions, Table 1.

The number of initiating groups present on the LBG backbone was controlled by the ratio of **4** to the saccharide repeat units, which each contain three hydroxyls. The balance of the hydrolysis of **4** and ester formation appears to lead to a non-linear relationship.

Table 1 Reaction conditions for LBG macroinitiator synthesis and control of the ratio of initiating sites per sugar ring (as determined by ^{1}H NMR spectroscopy after acid degradation)

Sample	Molar equivalents of sugar hydroxyl	Molar equivalents of BIBA/CDI	Molar ratio initiator/sugar repeat unit
LBG1	3	2/2	1/6
LBG2	3	1/1	1/15
LBG3	3	0.5/0.5	1/33
LBG4	3	0.25/0.25	1/100

Table 2 SEC-MALLS characterisation of various Locust Bean Gum macroinitiators. * LBG as received

Sample	Molar ratio of initiator/sugar repeat unit	M_n	M_w	Polydispersity (M_w/M_n)
	0*	917,450	1,016,500	1.11
LBG1	1/6	21,000	43,175	2.06
LBG2	1/15	36,050	64,580	1.79
LBG3	1/33	56,160	102,500	1.83
LBG4	1/100	167,400	333,800	1.99

The molecular weight of the macroinitiators was analysed by size exclusion chromatography on an instrument fitted with a multi-angle laser light scattering detector (SEC-MALLS) using an eluent of 0.05 M $NaNO_3$–1 M NaOH–20% MeOH. Under these conditions, it was possible to determine number average (M_n) and weight average (M_w) molecular weights and the polydispersity of the polysaccharides before and after macroinitiator synthesis. LBG as supplied was of very high molecular weight (M_n = 917,450). The reaction conditions employed here, however, had a significant effect on the polysaccharide molecular weight, which seems to be linked with the concentration of **4**, but is probably a combination of factors including temperature and shear, Table 2.

The molecular weight decrease during macroinitiator LBG4 synthesis was studied over time and indicated a significant decrease after 50 minutes reaction to an observed M_n = 590,000 (M_w/M_n = 1.15). After 1 hour, a further decrease was seen to M_n = 249,000 (M_w/M_n = 1.73) and after 3.5 hours, the macroinitiator had decreased to M_n = 196,000 (M_w/M_n = 2.00).

Aqueous ATRP may be carried out under a range of conditions including using low levels of water in alcoholic media, but ATRP conducted in 100% water rarely produces good molecular weight control or low polydispersity due to high polymerisation rates. The LBG macroinitiators, however, were fully soluble in demineralised water and were used at ambient temperature in 100% water at a concentration of 10% w/w. A molar ratio of initiating group/Cu(I)Br/bipyridyl (bipy) ligand was maintained at 1 : 1 : 2, considering macroinitiator M_n and the initiatior/sugar unit ratio.

Various comonomers have been studied and a number of different degrees of polymerisation (DP), ranging from 10–75 monomer units per graft, have been targeted. Water-soluble methacrylate monomers included in the study were sodium methacrylate (NaMA), **6**, 2-dimethylaminoethyl methacrylate (DMAEMA), **7**, 2-hydroxyethyl methacrylate (HEMA), **8**, 2-(sulfoxy)ethyl methacrylate (SEM), **9**, and monomethoxy poly-(ethylene glycol methacrylate) (PEGMA), **10**. Two styrenic monomers, 4-styrene sulfonic acid sodium salt (SSA), **11**, and 4-vinyl-benzoic acid sodium salt (VBA), **12**, and one acrylamide monomer, 2-acrylamido-2-methyl-1-propanesulfonic acid sodium salt (AMPS), **13**, were also evaluated.

Typically, graft copolymerisations using the LBG-macroinitiators were carried out by dissolving the monomer of choice and the macroinitiator in water followed by degassing the solution with N_2 followed by addition of the Cu catalyst and the bipy ligand. The solutions became significantly viscous after a few minutes indicating rapid polymerisation, however, detailed kinetics studies proved to be difficult in this complex mixture. Purification of the graft copolymers was accomplished by removal of the catalytic copper using a silica or basic alumina column after diluting the

Table 3 Locust Bean Gum graft copolymers using PEGMA monomers of varying ethylene glycol chain length (* – polymer solution viscosity too high to achieve accurate NMR data)

Monomer $PEGMA_{(x)}$	Macroinitiator (initiator/sugar)	Graft DP_n (theory)	^1H NMR calculated graft DP_n		M_n, M_w/M_n (SEC-MALLS) of graft copolymer
			D_2O	D_2O–DCl	
$PEGMA_{(7)}$	LBG2 (1/15)	3	7	5	53770, 1.60
		10	12	7	141150, 1.90
	LBG3 (1/33)	3	3	4	96200, 1.50
		10	15	10	106150, 1.60
	LBG4 (1/100)	3	4	5	61190, 1.60
		10	13	10	116200, 1.50
$PEGMA_{(22)}$	LBG2 (1/15)	6	6	3	295250, 3.50
	LBG3 (1/33)	6	7	7	118800, 1.60
	LBG4 (1/100)	6	7	6	102250, 1.60
$PEGMA_{(45)}$	LBG2 (1/15)	3	—*	3	238450, 3.10
	LBG3 (1/33)	3	—*	6	84685, 1.80
	LBG4 (1/100)	3	—*	5	109550, 1.50

polymerisation mixture with water. Even after high dilution, some polymer solutions remained highly viscous and a modified Cu removal procedure, involving rolling the polymer solution with silica or basic alumina and centrifugation, was used. The resulting colourless solutions were freeze-dried to give the dry polymer as a white powder.

Characterisation of the graft copolymers was conducted using ^1H NMR spectroscopy and SEC-MALLS. ^1H NMR was particularly useful in determining the DP of the grafts both before and after acid degradation. Previous characterisation of the macroinitiator was used to determine the initiator/sugar ratio, therefore, identification of the ^1H NMR resonances specifically attributable to the polymer grafted to the polysaccharide backbone allowed the calculation of the number average DP (DP_n) of the polymer graft. A comparison of the calculated graft DP_n, before and after acid degradation, of a series of PEGMA grafts, with varying ethylene glycol chain length, obtained from different macroinitiators is shown in Table 3.

The calculations of the DP_n of the grafted polymer chains by ^1H NMR show a good correlation from both "intact" and acid-degraded polysaccharide and are close to the target values. Also, the ethylene oxide chain length of the PEGMA monomer does not seem to hamper the polymerisation at these low DPs.

A similar evaluation was carried out on a series of monomers using LBG2 as the macroinitiator and a target DP_n = 50 repeat units. The polysaccharide backbone was not degraded for graft DP_n determination as ^1H NMR in D_2O was shown to be a reliable technique. NaMA, **6**, has been reported to polymerise using aqueous ATRP techniques, but to low DP; however, the LBG macroinitiator yielded a DP_n = 44, in good agreement with the targeted DP. DMAEMA, VBA, SSA and AMPS all polymerised equally well under these conditions (DP_n observed = 50, 70, 46 and 64, respectively). SEM proved difficult and only achieved 9 monomer units (target DP = 50). Due to solubility issues, a target DP_n of 10 units was attempted for HEMA with good results (DP_n obs. = 9).

SEC-MALLS analysis, Fig. 1, after graft copolymerisation shows a significant increase in the molecular weight of the polymer across the entire distribution. Also at all retention volumes, a higher molecular weight is observed, showing the branched and

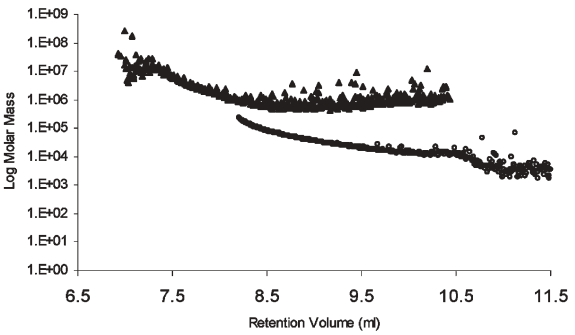

Fig. 1 Plot of molar mass *vs.* retention volume showing macroinitiator LBG2 (○) and the result of graft copolymerisation using styrene sulfonic acid monomer (▲).

compact nature of the copolymer relative to the starting macroinitiator.

In summary, we have demonstrated the first homogenous graft modification of LBG using controlled radical polymerisation in water at ambient temperature. Macroinitiators with varying molecular weight and number of initiating sites have been produced. The macroinitiators initiate a range of monomers under aqueous ATRP conditions (100% water) yielding high molecular weight graft copolymers with accurate targeting of graft length. Further work to investigate other polysaccharides is ongoing as are investigations of the mechanism of macroinitiator synthesis and graft copolymerisation. The authors wish to acknowledge the Royal Society for an Industry Research Fellowship (SPR).

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