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Graft copolymers of hydroxyethyl cellulose by a ‘grafting to’ method: $^{15}$N labelling as a powerful characterisation tool in ‘click’ polymer chemistry

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We demonstrate how $^{15}$N labelling can be used to probe the success of ‘grafting to’ processes, through the preparation of well-defined graft copolymers of hydroxyethyl cellulose by combining RAFT polymerisation and copper-catalysed azide-alkyne cycloaddition (CuAAC). Using synthesised alkyne-functionalised chain transfer agents, short-chain (DP=10) poly(N-vinylpyrrolidone) (PVP) and poly(N-isopropyl acrylamide) (PNIPAAm) were prepared in high conversion in a controlled manner ($\theta_m$ of ~1.4 and 1.2 respectively). Separately, partially $^{15}$N-labelled Ni-HEC was synthesised and characterised using solid state $^{13}$C, $^{15}$N CP-MAS NMR and FTIR spectroscopies. Alkyne-terminated PVP and PNIPAAm were grafted at different graft densities onto partially $^{15}$N-labelled Ni-HEC using the click reaction. The hybrid HEC-g-polymer materials were fully characterised using solid state $^{13}$C and $^{15}$N CP-MAS NMR and FTIR spectroscopies. While $^{13}$C and FTIR spectroscopies gave indirect or weak evidence of CuAAC coupling, the cycloaddition of the alkyne-terminated polymers with Ni-HEC was proven unambiguously by $^{15}$N solid state NMR spectroscopy. This indicates the utility of $^{15}$N labelling for probing the coupling efficiency of CuAAC reactions when employed in ‘grafting to’ processes with cellulosic substrates.

**Introduction**

With an annual production of $\sim 10^{11-12}$ tonnes$^{1-3}$, cellulose is the most abundant bio-polymer on Earth and the major compound in the cell wall of plants. Cellulose possesses a supra-molecular structure which results from the presence of two H-bonding networks. The intramolecular H-bonding network occurs between hydroxyl groups within the same chain whereas the intermolecular H-bonding results from the interaction of the hydroxyl groups between chains. These networks are responsible for the main drawback of cellulose, which is its poor solubility in both organic and aqueous solvents$^4$. This results in difficulties in processing cellulose, limiting its industrial applications. Chemical modification of cellulose is possible due to the presence of three hydroxyl groups at C2, C3 and C6 positions, resulting in derivatives such as hydroxyethyl cellulose (HEC), hydroxypropyl cellulose (HPC) and carboxymethyl cellulose (CMC) where the degree of substitution (DS) ranges from 0 to 3$^7$. HEC (Figure 1) is obtained by etherification with ethylene oxide and is defined by both the DS and the molecular substitution (MS), which represents the length of the ethyleneoxide side chain. This addition to the cellulose backbone disrupts the H-bonding networks resulting in solubilisation in polar solvents such as dimethylsulfoxide (DMSO) and water. HEC has been used extensively as an emulsifier, a stabiliser, thickeners and cosmetic film-formers in the formulation of hair and skin products$^8$.

![Fig. 1: Chemical structure of HEC](image_url)

A promising method for extending the range of industrial uses of cellulose is the preparation of graft copolymers. This allows the creation of new materials with the properties of synthetic polymers but which are obtained (at least in part) from a renewable feedstock. Three grafting approaches$^8$, 12, 13 can be employed: “grafting to”, “grafting from” and “grafting through”. The latter consists of polymerisation of monomeric...
species onto macro-monomers, and has been sparsely investigated in respect of cellulose derivatives. “Grafting from” is commonly used, where synthetic polymer chains are grown from sites on the cellulose backbone. The cellulose backbone can be activated via a range of processes including single electron transfer, hydrogen abstraction, irradiation or other methods. From the resulting macro-initiator sites, polymer chains can be grown producing the graft copolymer. The “grafting to” approach involves the chemical conjugation of a pre-prepared polymer to the cellulose backbone. The conjugation is possible through the presence on each polymer of complementary functional groups which undergo efficient coupling reactions such as thiol-ene, hetero-Diels-Alder or copper catalysed azide-alkyne cycloaddition (CuAAC).

Graft copolymerisation of cellulose derivatives has mainly been conducted by the “grafting from” method using a cellulose macro-initiator. Hansson et al. used ATRP to prepare cellulose-g-PMAA by grafting from a macroinitiator immobilised on a solid cellulose substrate. Semsarlar et al. were the first to report the use of RAFT in combination with a cellulose material; a hydroxypropyl cellulose (HPC) macro-CTA was used in the RAFT polymerisation of N-isopropylacrylamide (NIPAAM) and ethyl acrylate. Compared to the “grafting from” approach, however, “grafting to” has the significant advantage that it allows the full characterisation of both the grafts and the cellulose backbone prior to the coupling reaction. This method has been used to prepare a range of cellulose graft copolymers. Hansson et al. coupled alkyneterminated PMMA to a solid cellulose substrate, prior-functionalized with azide groups, using CuAAC ‘click’ chemistry. Goldman et al.11 grafted RAFT-polymerised poly(isobornyl acrylate) to a cellulose surface using a heteroDiels-Alder cycloaddition and recently, Xiao et al.99 used RAFT polymerisation in combination with a thiol-ene ‘click’ reaction to prepare methyl cellulose-g-poly(vinyl acetate).

In our work we sought to prepare well-defined graft copolymers of HEC using a homogeneous (solution) coupling approach. We chose RAFT polymerisation to prepare synthetic polymers because of its versatility in the range of monomers that are able to be polymerised in a controlled manner. Alkyneterminating polymer chain transfer agents suitable for preparing ‘clickable’ polymers from a range of monomers including N-vinylpyrrolidone, vinyl acetate, styrene and n-butyl acrylate, were employed. N-vinylpyrrolidone (NVP) was chosen to exemplify our approach because it is a challenge to polymerise by other CRP methods, such as ATRP. In order to demonstrate the versatility of our method, alkyneterminated PNIPAM was also prepared by RAFT polymerisation. Copper catalysed azide-alkyne cycloaddition (CuAAC) was chosen to ‘click’ alkyneterminated polymers to azido-HEC (N3-HEC). The HEC derivatives had limited solubility in common solvents, consequently characterisation was performed mainly using solid state methods. Indirect or weak confirmation of the CuAAC reaction was provided by solid state 13C NMR and FTIR spectroscopic investigations of the poorly soluble graft copolymer products. Partial 15N-labelling of N3-HEC, however, allowed unambiguous verification of the coupling reaction using solid state 15N NMR spectroscopy and is therefore a powerful tool to confirm the success of ‘grafting to’ processes involving CuAAC reactions with cellulose substrates.

Experimental

Materials and methods

All chemicals were purchased from Sigma Aldrich and were used as received. Solution state NMR spectra were recorded using a Bruker Advance 400 spectrometer at 400.13 MHz (1H) and 100.60 MHz (13C). For solid state NMR spectroscopy, a Varian VNMR spectrometer with a 9.4 T magnet was used and the 13C (100.56 MHz) and 15N (40.527 MHz) experiments were run using the cross polarisation method. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer. Molecular weight data were obtained using triple detection size exclusion chromatography (SEC) on a Viscotek TDA 302 with refractive index, viscosity and light scattering detectors and 2 x 300 mL PLgel 5 μm mixed C columns. Dimethylformamide (DMF) was used as the eluent at a flow rate of 1.0 mL/min and at a constant temperature of 70 °C.

Synthesis of azido-hydroxyethyl cellulose (N3-HEC)

The procedure followed that reported in the literature. In a round-bottomed flask fitted with a condenser, 2-hydroxyethyl cellulose (Mw = 90 000 g/mol, MS = 2.5, 2.5 g, 9.19×103 mol, 1 eq), sodium azide (3.8 g, 5.85×102 mol, 6 eq.) and 15N-labelled sodium azide Na15N3 (0.1 g, 1.54×10-3 mol, 0.1 eq) were dissolved in DMF (100 mL). The mixture was heated to 80 °C for 1 h in order to dissolve HEC. The mixture was cooled to room temperature and carbon tetrabromide (14.3 g, 4.31×102 mol, 5 eq) was added. Triphenylphosphine (11.8 g, 4.50×102 mol, 5 eq) dissolved in DMF (12.5 mL) was added carefully to the HEC mixture. The reaction was then left for 24 h at room temperature under magnetic stirring. The product was precipitated by addition of toluene (500 mL) and collected by filtration. The solid was dissolved in DMF (50 mL) and re-precipitated in diethyl ether (500 mL). After being filtered, the solid was washed with acetone (100 mL) and dried under vacuum at 50 °C overnight. The product 2 was obtained as a light yellow solid in quantitative yield (3.1 g). The product was characterised using solid state 13C, 15N CP-MAS NMR and FT-IR spectroscopies.

Synthesis of O-ethyl S-prop-2-ynyl carbonodithiolate (5)

The procedure follows that reported in the literature. In a one-neck round-bottomed flask, propargyl bromide solution 3 (80% wt. in toluene, 1.02 g, 8.57×103 mol) and potassium ethyl xanthenate 4 (1 g, 6.24×10-3 mol) were dissolved in THF (10 mL). The flask was covered with aluminium foil and the reaction was run overnight at room temperature. THF (100 mL) was added and the mixture was filtered to remove KOH. The
excess solvent was evaporated and distilled water (10 mL) was added to the residues. The product was extracted with diethyl ether (3×30 mL). The diethyl ether was removed and the final product 5 was purified via column chromatography using pentane as eluent and dried under vacuum overnight. The product was obtained as a pale yellow oil in a yield of 48% (0.48 g). $^{1}H$ NMR (400 MHz, DMSO-$d_{6}$): $\delta_{H}$ (ppm) 0.8 (t, J =7.0 Hz, 3H, CH$_3$-CH$_2$-O), 1.95-1.66 (m, 20H, CH$_2$-(CH$_2$)$_{10}$), 1.6 (s, 6H, -S-(CH$_2$)$_{15}$), 2.4 (t, 1H, J =2.4 Hz, -C=CH), 3.2 (t, 2H, J =7.5, -CH$_2$-S-), 4.6 (d, 2H, J =2.5 Hz, -O-CH$_2$); $^{13}$C NMR (100 MHz, CDC$_1$_$_3$): $\delta_{C}$ (ppm) 14.3 (CH$_3$), 22.8 (CH$_3$-CH$_2$), 25.4 (C-(CH$_3$)$_2$), 28-32 (9C, CH$_3$-CH$_2$-(CH$_2$)$_{10}$), 37.1 (-CH$_2$-S-), 53.5 (-O-CH$_2$), 55.2 (-C-(CH$_3$)$_2$), 75.2 (-C=CH), 77.4 (-C=CH), 172.5 (-C=O).

### RAFT polymerisation of N-vinylpyrrolidone

In a one-neck round-bottomed flask, O-ethyl S-prop-2-ynyl carbonodithioate 5 (0.50 g, 3.05×10$^{-3}$ mol, 1 eq.), AIBN (0.35 g, 2.13×10$^{-3}$ mol, 0.7 eq.) and N-vinyl-pyrroldione 9 (3.44 g, 3.09×10$^{-3}$ mol, 10 eq.) were dissolved in toluene (20 mL). The mixture was purged under N$_2$ for 10 min. The flask was sealed and the reaction was run overnight at 70 °C. The mixture was then cooled to room temperature and the product was precipitated in diethyl ether. The product was collected by filtration and dried overnight under vacuum at 40 °C. The product 10 was obtained as a white powder in quantitative yield (3.30 g). The polymer was characterized using solution state $^{1}H$ NMR spectroscopy and SEC. NMR spectra were recorded in CDC$_1$_$_3$ and the number average molecular weight (M$_{n}$) and the dispersity (D$_{n}$) were determined using SEC with conventional calibration (PMMA standards). Solid state $^{13}$N CP-MAS NMR (40.52 MHz): $\delta$N (ppm) -253.8.

### RAFT polymerisation of N-isopropyl acrylamide

In a one-neck round-bottomed flask, alkene-terminated CTA 8 (0.4 g, 9.93×10$^{-3}$ mol, 1 eq.), N-isopropyl acrylamide 11 (1.12 g, 9.90×10$^{-3}$ mol, 10 eq.), and AIBN (0.02 g, 1.21×10$^{-4}$ mol, 0.12 eq.) were dissolved in 1,4-dioxane (10 mL). The flask was purged under N$_2$ for 15 min and, once sealed, the polymerisation was run overnight at 60 °C. The polymer was precipitated in hexane (100 mL) and recovered by filtration. The product 12 was dried overnight under vacuum at 40 °C and was obtained as a white powder in a yield of 87% (1.12 g). The polymer was characterised using solution state $^{1}H$ NMR spectroscopy and SEC. NMR spectra were recorded in CDCl$_3$ and the M$_{n}$ and D$_{n}$ were determined using SEC with conventional calibration (PMMA standards).

### Copper-catalysed azide-alkyne cycloaddition (CuAAC) between N$_3$-HEC and 5

In a one-neck round-bottomed flask, N$_3$-HEC 2 (0.5 g, 1.7×10$^{-3}$ mol, 1 eq.), O-ethyl S-prop-2-ynyl carbonodithioate (0.7 g, 4.4×10$^{-3}$ mol, 3 eq.) 5, sodium L-ascorbate (0.24 g, 3.3×10$^{-4}$ mol, 0.2 eq.), copper (II) sulfate pentahydrate (0.15 g, 1.7×10$^{-4}$ mol, 0.1 eq.) and N,N,N,N'-tetramethylethylenediamine (0.07 g, 1.7×10$^{-4}$ mol, 0.1 eq) were dissolved in DMF (10 mL). The flask was heated at 30 °C for 24 h, the mixture was cooled to room temperature and the products were precipitated with chloroform (100 mL). The solid was then filtered and washed in acetone (~20 mL) under stirring in order to remove the unreacted RAFT agent. The product 13 was then filtered and dried overnight under vacuum at 50 °C. The product was obtained in near quantitative yield (0.7 g). The macro-CTA was characterised using solid state ($^{13}$C and $^{15}$N) CP-MAS NMR spectroscopies and FTIR spectroscopy.

The same procedure has been repeated without the use of copper sulfate pentahydrate in order to investigate the regioselectivity of the reaction. The resulting product obtained in a yield of 66% (0.33 g) was characterised using ($^{13}$C and $^{15}$N) CP-MAS NMR and FTIR spectroscopies.

### Copper-catalysed azide-alkyne cycloaddition (CuAAC) between N$_3$-HEC and alkene-terminated polymers

In a round-bottomed flask fitted with a drying tube, N$_3$-HEC 2 (0.5 g, 1.7×10$^{-3}$ mol, 1 eq.), alkene-terminated poly(N-vinylpyrrolidone) 10 (2 eq, 0.3 eq and 0.5 eq), sodium L-ascorbate (0.57 g, 2.88×10$^{-3}$ mol, 2 eq.), copper (II) sulfate pentahydrate (0.36 g, 1.44×10$^{-3}$ mol, 1 eq.) and N,N,N,N',N'-tetramethylethylenediamine (0.17 g, 1.46×10$^{-3}$ mol, 1 eq) were dissolved in DMF (20 mL). The flask was heated at 30 °C for 24 h. The mixture was cooled to room temperature and polymeric materials were precipitated with diethyl ether (200 mL). The solid was then collected by filtration and was washed for 24 h in water (~20 mL) under stirring in order to remove the unreacted PVP chains. The product 14 was then filtered and dried overnight under vacuum at 50 °C. The product was obtained in a yield of 40% mass. The graft-copolymers were

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characterised using solid state $^{13}$C and $^{15}$N CP-MAS NMR and FTIR spectroscopies.

The same procedure has been followed for coupling PNIPAAm 12 (1.06 g, 6.91×10^{-4} mol, 3 eq.) to N$_3$-HEC 2 (0.06 g, 2.02×10^{-4} mol, 1 eq.) and the resulting product 15 was obtained in quantitative yield (0.47 g). The polymer was characterised using solid state $^{13}$C, $^{15}$N CP-MAS NMR and FTIR spectroscopies.

**Results and discussion**

To prepare HEC graft copolymers by a ‘grafting to’ strategy using CuAAC coupling, we require HEC with one coupling partner in the side-chain and polymers with the other coupling partner as an end group. We decided to install the alkyne at the polymer chain end using alkyne-functionalised chain transfer agents. These would be coupled to azide-substituted HEC (N$_3$-HEC).

**Synthesis of azido-hydroxyethyl cellulose (N$_3$-HEC)**

N$_3$-HEC was prepared from HEC in a one-step procedure in the presence of carbon tetrabromide and triphenyl phosphine (Scheme 1)\textsuperscript{36}. In order to aid the detection of nitrogen by $^{15}$N NMR spectroscopy, sodium azide was doped with Na$^{15}$N$_3$.

The resulting azido-HEC product was found to have poor solubility in common solvents, therefore characterisation was performed in the solid state. The solid state $^{13}$C NMR spectrum (Figure 2b) shows signals at ~103 ppm, 83 ppm, 74ppm, 71 ppm and 52 ppm. The signals at ~103 ppm and 81 ppm are typical of HEC and are assigned respectively to the anomeric carbon (C1) and the carbon at the C4 position. The signals $\delta$C ~74 ppm are assigned to the set of carbons at C2, C3 and C5 positions and the peak $\delta$C ~71 ppm is assigned to the carbons of the ethylene oxide side chain. The signal at ~52 ppm is assigned to the –CH$_2$-N$_3$ and the loss of the signal at ~62 ppm in the HEC spectrum commonly assigned to the C6 (R=H) and the final carbon of the ethylene oxide side chain indicates complete functionalization of the primary alcohol of HEC with Na$^{15}$N$_3$.

The solid state $^{15}$N NMR spectrum (Figure 2b) shows two signals at ~310 and ~170 ppm which are characteristic respectively of –CH$_2$-N$_3$=N$_3$ and –CH$_2$-N$_3$=N$_3$\textsuperscript{+}. The labelled Na$^{15}$N$_3$ is labelled at N$_3$ and N$_3$ only, and this explains why N$_3$ was not detected. In the cross polarisation method, the detection of nitrogen is obtained from the transfer of energy from protons to the nitrogen. Thus, the more protons environments, there are near to the nitrogen and the closer these proton are to the nitrogen, the easier will be their detection. In N$_3$-HEC, N$_3$ is directly attached to a methylene group which...
represents also the closest source of protons for Nα. The excitation energy received by Nα is greater than that received by Nγ, resulting in an easier detection of Nα which is demonstrated by a higher intensity of the signal δH ~310 ppm assigned to Nα. The FTIR spectrum of N3-HEC (Figure 2c) shows a strong absorbance band at 2100 cm⁻¹, characteristic of the presence of an azide group and a decrease of the absorbance band at 3400 cm⁻¹ indicating the loss of some hydroxyl groups, reinforcing the evidence of the functionalization of the primary alcohol with sodium azide.

**Preparation of alkyne-terminated polymers**

The chain transfer agent (CTA) O-ethyl-S-prop-2-ynyl carbonodithiolate (5) was prepared following a reported procedure. Propargyl bromide was reacted with potassium ethyl xanthogenate overnight at room temperature to obtain 5 in a yield of 50% (Supplementary Information, Scheme S1). The CTA 5 was then used to polymerise N-vinylpyrrolidone (NVP) in a controlled manner to give PVP chains with alkyne end groups (Supplementary Information, Scheme S3). In the literature, both xanthate and diithiocarbamate chain transfer agents were shown to be good candidates for polymerising NVP, a monomer that is a member of the “less activated monomers” (LAM) class described by Keddie et al. Furthermore, Patel et al. and Akeroyd et al. reported the preparation of alkyne-terminated RAFT agents which were used for preparing “macro-RAFT agents” or clickable polymers respectively. The ratio RAFT agent : initiator (AIBN) was optimised to obtain relatively high conversion and good control of the polymerisation, and we found that 1:0.7 was a good compromise, giving a monomer conversion and a D_M of 80% and 1.4 respectively. The degree of polymerisation (DP_{target} =10) was kept low in order to facilitate the spectroscopic detection of the HEC backbone and PVP in the final graft-copolymer HEC-g-PVP. At the end of the reaction, 80% monomer conversion was determined and PVP was isolated by precipitation with diethyl ether in near-quantitative yield. The polymer was then characterised using NMR spectroscopy and SEC. The ¹H NMR spectrum of PVP (Supplementary Information, Figure S1) shows signals characteristic of the protons of the pyrrolidone ring as well as peaks that can be assigned to the chain end -CH₂ and the methylene adjacent to the alkyne. Furthermore, the signal at δ_H ~4.6 ppm is assigned to the non-alkyne end group CH₂-O. The DP was estimated using the integral of the signal at δ_H ~4.6 ppm, which gave an Mₙ NMR value of 1,500 g/mol. This is in good agreement with the targeted DP of 10.

The SEC results (Supplementary Information, Figure S2) indicate a number average molecular weight (Mₙ SEC) of 1 180 g/mol and a dispersity (D_M) of 1.4, calculated with conventional calibration using PMMA standards. The Mₙ SEC value is not representative of the true value because of a non-negligible difference of the hydrodynamic volume of PMMA standards and PVP chains. However, the D_M value is characteristic of the true molecular weight distribution. The D_M value of 1.4 indicates a relatively good control of the polymerisation, given the low DP.

An alkyne-terminated trithiocarbonate 8 was synthesised from CTA 6 and propargyl alcohol in a yield of 87% (Supplementary Information, Scheme S2). NIPAAM was successfully polymerised using 8 at a 1:0.12 ratio of initiator to chain transfer agent (Supplementary Information, Scheme S4). The alkyne-ended pNIPAAM was obtained with a monomer conversion of 90% determined by ¹H NMR spectroscopy. In the ¹H NMR spectrum (Supplementary Information, Figure S3), some of the transfer chain signals are detected with the peak at δ_H ~0.8 ppm assigned to the methyl group of the chain end, δ_H ~1.2 ppm to the gem-dimethyl groups, δ_H ~1.8 ppm to the -C≡CH, δ_H ~3.2 ppm to the -CH₂-S-, δ_H ~4.6 ppm to the -O-CH₂- and δ_H ~6 ppm to the -NH group. From the integration of the signal of the end group at δ_H ~4.6 ppm compared to the methine group at δ_H ~3.9 ppm characteristic of PNIPAAM, a degree of polymerisation (DP_{NMR}) of ~10 was estimated and this corroborated with the calculated monomer conversion. The D_M measured by SEC was found to be 1.2, confirming the control of the polymerisation. Because of a very low targeted DP, the calculation of the molecular weight using SEC was not reliable regardless of the detector used. However, the confirmation of a narrow molecular weight distribution permitted the use of the calculated DP_{NMR} to give an approximation of the molecular weight as ~1,400 g/mol. Xanthate CTA 5 did not give good control of NIPAAM RAFT polymerisation ((D_M =1.5; Supplementary Information, Figure S4)

**Copper Catalysed Azide-alkyne Cycloaddition (CuAAC)**

In order to test the efficiency of the CuAAC reaction, coupling was attempted initially with alkyne-CTA 5 (Supplementary Information, Scheme S5). It was expected that the low molar mass of 5 would aid characterisation of the click product. CTA-functionalized HEC was prepared via CuAAC in 100% yield assuming complete coupling. Again due to poor solubility of the coupling product in deuterated NMR solvents, it was characterised in the solid state using FTIR and solid state NMR spectroscopies.

The solid state ¹³C NMR spectrum (Figure 3a) shows a large band of peaks between 71 and 81 ppm which are assigned to the carbons of the HEC backbone. The peak at 103.2 ppm is assigned to the carbon at the C1 position, and the peak at 51.9 ppm is assigned to the carbon directly attached to the nitrogen of the triazole ring. Comparing the product to the N3-HEC spectrum (Figure 2), the chemical shift of this carbon is the same as that of CH₂-N₃. However, the click reaction is demonstrated by the presence of peaks at 124.0 and 123.2 ppm which are assigned to the carbons in the triazole ring. Furthermore, the peak at 15.0 ppm and the broad peak at 32.4 ppm are assigned respectively to the methyl and methylene of the RAFT agent. The FTIR spectrum (Figure 3b) showed a strong decrease of the absorption band at 2100 cm⁻¹ assigned to the azide groups, which provides indirect evidence of the functionalization reaction (disappearance of azide groups). The
coupling between the azide and the transfer agent however appears to not be complete.

To provide further evidence of the success of the ‘click’ reaction, solid state $^{15}$N NMR spectroscopy was employed. In the $^{15}$N NMR spectrum (Figure 3c), only one peak at $\delta_N \sim$134 ppm is present and is assigned to the $N_a$ of the triazole cycloaddition product.

The large change in the chemical shift of $N_a$ from -310 ppm to -134 ppm suggests complete reaction of $N_3$ with the alkyne group of the transfer agent, however, because the FTIR spectrum showed the presence of unreacted $N_3$, we suppose that the peak at -310 ppm corresponding to unreacted $N_a$ was too weak and thus was undetectable by NMR. Compared to the spectrum of $N_3$-HEC (Figure 2), the ratio of the signal of $N_a$ to noise is lower due to the change of the environment in terms of protons and the change in the symmetry of the environment. The peak present at -170 ppm in the $^{15}$N spectrum of $N_3$-HEC (Figure 2) and assigned to $N_a$ is absent from the $^{15}$N spectrum of the macro-CTA because the ratio of signal $N_a$ to noise has decreased, resulting in an inability to detect it. This peak was expected to shift to approximately -20 ppm according to studies carried by Corredor et al.\textsuperscript{45} $N_3$-HEC was therefore quasi-completely functionalised with the RAFT agent using a CuAAC reaction resulting in the formation of a CTA-functionalised HEC backbone. This allows us to identify the correct conditions for the click reaction and also to determine the $^{15}$N NMR chemical shifts of the triazole product, which will be of great value in confirming the grafting of alkyne-ended polymers to $N_3$-HEC.

The CuAAC reaction was next used to couple PVP$^{10}$ to $N_3$-HEC, forming the graft-copolymer HEC-g-PVP$^{10}$ (Supplementary Information, Scheme S5). A chain length of 10 repeat units was chosen because this aided the characterisation of the graft copolymer and the properties of HEC should be retained. To examine the scope of the grafting process, ratios of PVP chain to azide groups equal to 1:5, 1:3 and 1:1 were chosen. The graft-copolymer and possibly some unreacted PVP$^{10}$ chains were precipitated with diethyl ether. Ungrafted PVP$^{10}$ chains were then separated from HEC-g-PVP$^{10}$ by extensive washing in water (PVP$^{10}$ chains are water soluble whereas HEC-g-PVP$^{10}$ is not). Based on the theoretical ratio of PVP chains to azide groups, HEC-g-PVP$^{10}$ was obtained in a yield of ~40%.

In the $^{13}$C NMR spectrum of HEC-g-PVP$^{10}$ with a ratio of PVP to $N_3$ equal to 1:5 (Figure 4b), peaks characteristic of both HEC and PVP were detected. The peak at 51 ppm is assigned to the carbon at the C10 position, the large band between 60 ppm to 90 ppm is assigned to the carbons of the HEC ring and the ethyleneoxide side chain and the peak at 104 ppm is assigned to the anomic centre (C1) (structure numbering given in Supplementary Information, Figure S5). The presence of PVP is demonstrated by the peaks at 19.3 ppm, 32.6 ppm, 43.8 ppm and 176.7 ppm which are assigned respectively to the pyrrolidone ring and the carbonyl group. When the ratio of PVP to $N_3$ is increased to 1:3 (Figure 4c), the intensity of the peaks assigned to PVP increases whereas the intensity of the peaks characteristic of HEC decreases, due to the increase of the density of grafting of HEC with PVP$^{10}$. Increasing the graft-density to attempt to achieve complete functionalization of $N_3$-HEC with PVP resulted in an almost complete disappearance of HEC signals (Figure 4d). The triazole carbons resulting from the cycloaddition between PVP$^{10}$ and $N_3$-HEC were not
detected because of their low concentration within the structure. The NMR experiments indicate the presence of PVP and HEC in the product, and the signals of the HEC backbone decreased with increasing grafting density. However, definitive evidence of the coupling is not demonstrated using these NMR experiments.

To try to demonstrate more clearly the CuAAC coupling between PVP and N₃-HEC, partially ¹⁵N-labelled N₃-HEC was prepared. In the ¹⁵N NMR spectrum of N₃-HEC (Figure 6a), the two peaks at ~310 and ~170 ppm are assigned to N₀ and N₁. When PVP₁₀ was coupled to N₃-HEC at a ratio PVP:N₃ equal to 1:5 (Figure 6b), a peak at ~256.3 ppm appeared which is assigned to the unlabelled nitrogen in the pyrrolidone ring (the ¹⁵N signal for PVP₀ occurs at ~253.8 ppm). Increasing the PVP:N₃ ratio to 1:3 (Figure 6c) resulted in the detection of an additional signal at ~140 ppm which is assigned to N₀ after its cyclisation with PVP₁₀. The peaks of unfunctionalized N₀ and N₁ are still present in the spectrum but their intensities are decreased compared to the signal of nitrogen in PVP, indicating an increase of the functionalization of N₃-HEC with PVP₁₀.

For the spectrum of HEC-g-PVP₁₀ prepared from a ratio PVP:N₃ equal to 2:1 (Figure 6d), two signals at ~256.3 ppm and ~140.4 ppm are detected and are assigned respectively to the nitrogen in the pyrrolidone ring and the triazole N₀ after the cycloaddition process. The intensity of the signal of N₁ in N₃-HEC decreased when the graft-density increased, however, no N₁ signal was detected after cyclisation. The complete loss of both signals at ~310 ppm and ~170 ppm, assigned respectively to N₀ and N₁ in N₃-HEC, demonstrates complete coupling between N₃-HEC and PVP₁₀ and this corroborates with the discussion of the ¹³C NMR and FTIR spectra of HEC-g-PVP₁₀.

In order to demonstrate the versatility of our grafting method, PNIPAAM₁₀ synthesised with an alkyne-terminated triiicarbonate CTA was coupled to N₃-HEC using identical conditions to those described in the previous section (Supplementary Information, Scheme S6). PNIPAAM peaks were detected in the solid state ¹³C NMR spectrum of the graft copolymer (Figure 7a). Additional weak peaks (Figure 7a inset) are observed when increasing the intensity of signals and these are assigned either to the cellulose backbone or the chain

Fig. 5. FTIR spectra of (a) N₃-HEC, (b) HEC-g-PVP₁₀ (1:5 PVP:N₃), (c) HEC-g-PVP₁₀ (1:3 PVP:N₃), (d) HEC-g-PVP₁₀ (2:1 PVP:N₃).

Fig. 6. Solid state ¹⁵N CP-MAS NMR spectra of (a) N₃-HEC, (b) HEC-g-PVP₁₀ (1:5 PVP:N₃), (c) HEC-g-PVP₁₀ (1:3 PVP:N₃), (d) HEC-g-PVP₁₀ (1:1 PVP:N₃). Theoretical density of grafting denoted by x and y where x = azido-HEC units and y = PVP grafted units. Inset above a) shows labelling of azide nitrogens.

Fig. 4. Solid state ¹³C CP-MAS NMR spectra of (a) N₃-HEC, (b) HEC-g-PVP₁₀ (1:5 PVP:N₃), (c) HEC-g-PVP₁₀ (1:3 PVP:N₃), (d) HEC-g-PVP₁₀ (1:1 PVP:N₃). Theoretical density of grafting denoted by x and y where x = azido-HEC units and y = PVP grafted units. Protons of the pyrrolidone ring of PVP are labelled k, l, m and n (see Supplementary Information for assignment).
transfer agent. The peaks at 14.7 ppm and 242.4 ppm are assigned to the methyl and thiocarbonate group of the RAFT agent. Furthermore, bands at ~71 and ~107 ppm are characteristic of the NMR signals commonly observed for cellulose materials (Figure 7b), and were assigned respectively to the set of carbons at C2 to C6 positions and to the anomeric centre (C1). The cycloaddition between PNIPAAM and N3-HEC was suggested by the presence of weak signals at ~125 and 143 ppm which are assigned to triazole ring carbons. The FTIR spectrum (Supplementary Information, Figure S6) showed a complete disappearance of the azide band suggesting successful reaction with the alkynyl group at the chain end of PNIPAAM.

The solid state $^{13}$C NMR spectrum (Figure 8a) of the graft-copolymers shows two signals -134.8 ppm and -244 ppm which are respectively assigned to the labelled nitrogen $^{15}$N$_3$ of N3-HEC after the cycloaddition and the unlabelled nitrogen of PNIPAAM. The difference in intensity between the two peaks is explained by the concentration of each within the structure; there is only one partially labelled N$_3$ per 10 nitrogens of PNIPAAM in the structure, resulting in a low intensity of the signal of N$_3$. Furthermore, the signals of N$_3$ and N$_2$ of N3-HEC (Figure 8b) disappeared after the cycloaddition, indicating complete functionalization.

**Conclusions**

Graft copolymers of hydroxyethyl cellulose (HEC) and either poly(N-vinylpyrrolidone) (PVP) or poly(N-isopropylacrylamide) (PNIPAAM) have been prepared by a ‘grafting to’ approach using CuAAC coupling. To achieve this, azido-substituted HEC and alkyne-terminated RAFT polymers were prepared. A degree of polymerisation of 10 was targeted for each polymer so that the final graft copolymer would retain a significant mass fraction of HEC after coupling. Both RAFT polymers were synthesised successfully with monomer conversions of 80-90% and $D_M$ values ranging from 1.2 (PNIPAAM) to 1.4 (PVP). HEC-g-PVP$_{10}$ and HEC-g-PNIPAAM$_{10}$ hybrid materials with different graft densities were prepared by CuAAC coupling using different ratios of synthetic polymer to azide groups. Solid state $^{13}$C NMR and FTIR spectroscopies provided weak or indirect evidence of coupling of RAFT polymers to HEC, however the use of $^{15}$N-labelled N3-HEC and $^{15}$N solid state NMR spectroscopy allowed unequivocal demonstration of the successful CuAAC reaction. Our strategy for preparing graft-copolymers of cellulose is efficient and produces extremely well-defined hybrid materials by combining RAFT polymerisation and CuAAC. Furthermore, we demonstrate that $^{15}$N labelling and solid state $^{15}$N NMR spectroscopy is an effective tool in probing CuAAC reactions involving poorly soluble polymer substrates.

**Notes and references**

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Electronic Supplementary Information (ESI) available: schemes showing synthesis of CTAs, polymers and graft copolymers; characterisation of PVP and PNIPAAM; and FTIR spectrum of HEC-g-PNIPAAM. See DOI: 10.1039/b000000x/
This is.entry includes a list of references for various topics such as polymer chemistry, drug delivery, and controlled/living radical polymerization. The references are cited in a variety of journals and date back to 1963, indicating a comprehensive coverage of the field. The list is arranged in ascending order by year, starting from 1963 and ending in 2012.