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Modification of dextran using click-chemistry approach in aqueous media

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ABSTRACT

In this paper, dextran was modified using click-chemistry. Each reaction step was done under aqueous conditions, including the introduction of azide functionalities to the backbone of the polysaccharide. The reaction consisted of the synthesis of 1-azido-2,3-epoxypropane, which was etherified onto the backbone of the polysaccharide using base-catalysis in water/isopropanol mixture at ambient temperature. The achieved degree of substitution (DS) of azide groups in dextran was up to 0.20. Alkyne-end-functionalized poly(ethylene glycol) monomethyl ether (PEG-MME) was then grafted onto the synthesized azide-functionalized dextran having a DS of 0.08 or 0.16 by copper-catalyzed azide–alkyne cycloaddition (CuAAC). In the case of the lower DS valued dextran, a quantitative reaction of the azides was achieved within an hour, whereas the CuAAC-reaction of dextran having DS = 0.16 yielded a DS = 0.10 of grafted poly(ethylene glycol). Prolonging the reaction time to 24 h did not further improve the conversion of azides, which may be due to steric factors.

1. Introduction

There is a growing interest towards renewable raw materials, such as polysaccharides, which with a slight chemical modification could be used in many applications. Due to their non-toxicity, biocompatibility and biodegradability, the applications may vary from paper quality enhancement chemicals (Maurer & Kearney, 1998) to more sophisticated applications such as gene-therapy vectors (Rinaudo, 2008).

In order to elegantly tailor the properties of natural-based polymers, "click"-chemistry offers a straightforward and efficient way to avoid multiple reaction and purification steps (Kolb, Finn, & Sharpless, 2001). Moreover, click-chemistry concepts, such as ambient reaction temperatures and high tolerance towards oxygen and water, conveniently match with the chemical needs for the modification of these natural polymers.

The most referred "click"-reaction employed in polymer synthesis is the copper-catalyzed azide–alkyne cycloaddition (CuAAC) which offers various possibilities to tailor polymer properties (Binder & Sachsenhofer, 2007, 2008; Fournier, Hoogenboom, & Schubert, 2007; Meldal, 2008; Rostovtsev, Green, Fokin, & Sharpless, 2002; Tornoe, Christensen, & Meldal, 2002). The utilization of CuAAC on the modification of polysaccharides has been reported in several publications (Bernard, Save, Arathoon, & Charleux, 2008; De Geest et al., 2008a, 2008b; Hafrén, Zou, & Córdova, 2006; Hasegawa et al., 2006; Liebert, Hänsch, & Heinze, 2006; Schatz, Louguet, Le Meins, & Lecommandoux, 2009; Tankam, Müller, Mischnick, & Hopf, 2007) However, a major challenge in the exploitation of CuAAC in the modification of polysaccharides is the difficulty of introducing the azide or alkyne functionalities necessary for the subsequent CuAAC-reaction. In most cases, dry reaction conditions and solvents, such as dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF), have to be used in order to introduce the required azide or alkyne groups. This is not necessarily suitable for all polysaccharides and in some occasions it would be more convenient to use water as a solvent in every modification reaction.

In this paper, synthesis of dextran-*g*-poly(ethylene glycol) was done using CuAAC. The azide functionalities needed for the reaction were introduced by the etherification of 1-azido-2,3-epoxypropane onto the backbone of the polysaccharide in water/isopropanol mixture using different NaOH concentrations and temperatures. The introduced azides where further used in the grafting of alkyne-end-functionalized poly(ethylene glycol) monomethyl ether (PEG-MME). In this way, aqueous reaction media and ambient temperatures could be used, avoiding tedious drying or solvent-exchange steps and solubility problems often encountered when working with polysaccharides. The obtained dextran derivatives were characterized by ^{1}H, ^{13}C NMR, DEPT-135 and Fourier transform infrared (FT-IR) spectroscopies as well as with dynamic light scattering (DLS) studies.

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Table 1
The effect of added NaOH on the obtained degree of substitution (DS) in the etherification reaction of dextran with 1-azido-2,3-epoxypropane.

Entry[a]	Added NaOH (mmol)	pH	DS theoretical	DS observed[b]	Conversion of epoxide[c] (%)	Reaction efficiency (%)
A	5	13	1.34	0.13	100	10
B	4	13	1.34	0.13	100	10
C	3	13	1.34	0.17	100	13
D	2	13	1.34	0.15	100	11
E	1	12	1.34	0.12	88	9
F	0	11	1.34	0.00	60	0

[a] Reaction time 21 h at 30 °C. 7.9 ml (3.2 mmol) of 1-azido-2,3-epoxypropane solution, 0.388 g of dextran (2.39 mmol of sugar-units) in 20 ml of H_2O.
[b] Calculated based on ^{13}C NMR analysis.
[c] Calculated based on ^{1}H NMR analysis.

2. Materials and methods

2.1. Materials

Dextran (average molecular weight 60,000 g/mol) and HNO_3 (65%) were obtained from Fluka Chemicals and used as received. Poly(ethylene glycol) monomethyl ether (molecular weight 500 g/mol) was from Fluka Chemicals and it was dried overnight at 30 °C under vacuum prior to use. Propargyl bromide (80 wt% in toluene), L-ascorbic acid (99%), $CuSO_4{\cdot}5H_2O$ (99%), epichlorohydrin (99%) and $NaNO_2$ (97%) were purchased from Sigma–Aldrich and used as received. NaN_3 (99%), acetic acid (100%) and NaOH (99%) were purchased from Merck and used as received.

2.2. Preparation of 1-azido-2,3-epoxypropane

The synthesis of 1-azido-2,3-epoxypropane was done starting from epichlorohydrin. The ring-opening reaction of the epoxide with azide-ion was done according to a slightly modified method (Fringuelli, Piermatti, Pizzo, & Vaccaro, 1999). Isopropanol (16.6 ml) and acetic acid (1.1 ml, 19.2 mmol) were mixed with a solution of NaN_3 (11.2 ml, 19.1 mmol) in water. Epichlorohydrin (1.0 ml, 12.8 mmol) was then added under stirring and the reaction was continued at 30 °C for 21 h, until ^{1}H and ^{13}C NMR analyses showed complete consumption of the epoxide. A water solution of $NaNO_2$ (1.8 ml, 6.5 mmol) was then added, followed by the dropwise addition of HNO_3 (0.9 ml, 13.0 mmol) to eliminate any excess azide-ions. The obtained solution of 1-azido-3-chloropropanol (yield 100% by ^{1}H and ^{13}C NMR analyses) was stored in dark at room temperature and used without further purification.

^{1}H NMR (D_2O, ppm): δ = 3.36–3.54 (CH_2–Cl), 3.56–3.73 (CH_2–N_3).

^{13}C NMR (D_2O, ppm): δ = 70.50 (C–OH), 53.92 (C–N_3), 46.69 (C–Cl).

The conversion of 1-azido-3-chloropropanol to 1-azido-2,3-epoxypropane was done just prior to use, by adding 1 ml of 5 M NaOH to every 7.9 ml of the prepared 1-azido-3-chloropropanol-solution and stirring the mixture for 5 min, until the pH dropped from approximately 14–12. The obtained epoxide-solution (yield 100% by ^{1}H and ^{13}C NMR analyses) was immediately used for the azidation of dextran.

^{1}H NMR (D_2O): δ = 2.77–2.87 and 2.89–2.99 (CH_2O), 3.21–3.42 (CH_2–N_3), 3.67–3.81 (CHO).

^{13}C NMR (D_2O): δ = 52.41 (C–N_3), 52.11 (CHO), 46.15 (CH_2O).

2.3. Azidation of dextran using 1-azido-2,3-epoxypropane

The introduction of azide groups onto the backbone of dextran was done as follows (Table 1, entry C): to a water solution of dextran (0.388 g, 2.4 mmol of sugar-units), 0.60 ml of 5 M NaOH solution was added under stirring, the total amount of water being 20 ml. 7.9 ml (3.2 mmol) of freshly prepared solution of 1-azido-2,3-epoxypropane was then added. The obtained colorless clear reaction mixture was stirred for 21 h at 30 °C, until ^{1}H NMR analysis showed complete consumption of the epoxide. The solution was then neutralized with acetic acid and the polysaccharide was purified twice by precipitation to 150 ml of ethanol. The white precipitate was collected and dried overnight at 40 °C under vacuum.

^{13}C NMR (D_2O): δ = 97.96, 73.72, 71.60, 70.47, 69.82, 65.66 (dextran C–O), 72.21, 69.62 (C–O), 53.42 (C–N_3).

FT-IR: (KBr): ν = 3378 (OH), 2927 (CH), 2108 (N_3).

2.4. Preparation of alkyne-end-functionalized poly(ethylene glycol)

Dried poly(ethylene glycol) monomethyl ether (PEG-MME) (5.00 g, 10.0 mmol) was dissolved in 10 ml of THF, previously dried with molecular sieves. NaH (0.300 g, 12.5 mmol) was added to the solution and the mixture was stirred at 30 °C under argon for 10 min until the formation of hydrogen gas ceased. Propargyl bromide (1.4 ml, 12.6 mmol) was then added dropwise and the reaction was continued for 2 h at 30 °C, after which isopropanol (0.5 ml) was added. The reaction mixture was centrifuged to remove any formed salts and then poured to cold (0 °C) hexane. The precipitated pale brown polymer was collected, dissolved in THF, precipitated again in cold hexane and dried overnight in vacuum at 40 °C. The achieved alkyne-functionalization was approximately 90% based on ^{1}H and ^{13}C NMR analyses, the yield being 85%.

^{1}H NMR (d_6-DMSO): δ = 4.20–4.10 (propargyl CH_2), 3.80–3.10 (PEG-MME).

^{13}C NMR (d_6-DMSO): δ = 80.31, 77.03 and 57.49 (propargyl ether), 71.3, 69.60 and 68.52 (PEG), 58.05 (methyl ether).

2.5. Grafting of PEG onto dextran using CuAAC

The azidated dextran (Table 2, entry O, 0.916 g, DS = 0.08, 0.19 mmol of azide functionalities) and excess of the alkyne-end-functionalized PEG (0.172 g, 0.31 mmol) were dissolved in 8 ml of water. A freshly prepared solution of $CuSO_4{\cdot}5H_2O$ (0.008 g, 0.031 mmol) and ascorbic acid (AAc) (0.011 g, 0.062 mmol) in 2 ml of water was added and the reaction was carried out for 1 h at 30 °C. The product was precipitated to 150 ml of ethanol, redissolved in water, precipitated in ethanol two more times and dried overnight at 40 °C.

^{1}H NMR (D_2O): δ = 8.73–8.36 (triazole), 5.33–5.15 and 4.35–3.70 (dextran), 4.02–3.92 (PEG-MME).

^{13}C NMR (D_2O): δ = 144.21, 126.37 (triazole), 98.09, 73.78, 71.76, 70.58, 70.03, 66.07 (dextran ether), 71.35, 69.91, 58.44 (PEG-MME).

2.6. Characterization

^{1}H, ^{13}C NMR and DEPT-135 spectra were recorded on a Varian Gemini 2000 300 MHz spectrometer in deuterium oxide (D_2O) or deuterated dimethyl sulfoxide (d_6-DMSO). A pulse width of 13.1 μs (90°), relaxation delay of 10 s and acquisition time of 3 s was used for ^{1}H NMR. For quantitative ^{13}C NMR, a 90° pulse width of 18.0 μs

Table 2
The effect of reaction conditions on the obtained degree of substitution (DS) in the etherification of dextran with 1-azido-2,3-epoxypropane.

Entry[a]	Temp. (°C)	AEP (mmol)	Dextran (g)	Sugar-units (mmol)	DS theoretical	DS observed[b]	Reaction efficiency[c] (%)
C	30[d]	3.2	0.388	2.4	1.34	0.17	14
G	30[d]	6.4	0.388	2.4	2.67	0.16	6
H	30[d]	6.4	1.038	6.4	1.00	0.20	20
I	55[e]	3.2	0.388	2.4	1.34	0.10	8
J	70[e]	3.2	0.388	2.4	1.34	0.12	9
K	70[e]	3.2	1.038	6.4	0.50	0.07	14
L	55[e]	6.4	1.038	6.4	1.00	0.17	17
M	70[e]	6.4	1.038	6.4	1.00	0.16	16
N	55[e]	3.2	1.038	6.4	0.50	0.07	14
O	55[e]	3.2	2.076	12.8	0.25	0.08	32
P	55[e]	6.4	2.076	12.8	0.50	0.13	26
Q	55[e]	9.6	2.076	12.8	0.75	0.19	25

[a] 3 mmol of added NaOH in 20 ml of H_2O, pH = 13.
[b] Calculated based on ^{13}C NMR analysis.
[c] 100% conversion of the epoxide based on ^{1}H NMR analysis.
[d] Reaction time 21 h.
[e] Reaction time 4.5 h.

was used, the relaxation delay and acquisition time being 6 s and 1.8 s. 5000 scans were accumulated for each sample, and the decoupler was gated on only during acquisition, in order to suppress the nuclear Overhauser effect. DEPT-135 analysis was performed using 140 Hz average J_{CH} bond coupling. The infrared-spectra were obtained with Nicolet Magna IR750 from KBr-pellets. Dynamic light scattering measurements were done using Zetasizer Nano ZS (Malvern Instruments). The samples were prepared to concentrations of 1 mg/ml and filtered through 0.45 μm filter.

3. Results and discussion

3.1. Introduction of azide functionalities to the backbone of dextran

The introduction of azide groups was done by the reaction of 1-azido-2,3-epoxypropane with dextran under alkaline conditions, a method similar to which is commonly employed for the hydroxypropylation of polysaccharides, e.g. starch, using epoxypropane (Tomasik & Schilling, 2004). In this way, azide groups necessary for the subsequent CuAAC-reaction were introduced in a relatively simply way, without solvent-exchange or drying steps involved in the synthesis. Dextran was used in all reactions due to its relatively simple structure and good water solubility.

The preparation of 1-azido-2,3-epoxypropane was done in two steps starting with the ring-opening of epichlorohydrin with azide-ion in the presence of acetic acid, giving 1-azido-3-chloropropanol, which in turn was converted to the epoxide-form with alkaline treatment in high yield (Fringuelli et al., 1999). Due to the known fact that low-molecular weight organic azides are potentially explosive substances, there were no attempts to purify or concentrate the obtained 1-azido-2,3-epoxypropane solution, but it was used directly for further reactions.

Table 1 shows the effect of the amount of added NaOH to the obtained degree of substitution (DS) for the azide groups in dextran. As expected, the azidation does not occur when no additional NaOH is used, since the amount of NaOH from the preparation of 1-azido-2,3-epoxypropane alone is insufficient to elevate the pH value. In this case, there is not enough reactive alkoxides formed on the backbone of the polysaccharide that can undergo the ring-opening of the epoxide. Adding sodium hydroxide raises the pH, allowing the azidation to take place, and a maximum value of DS = 0.17 is observed when 3 mmol of NaOH is added. Adding more base lowers the obtained DS, probably due to the increasing proportion of the competitive reaction, that is, the direct addition of hydroxide ion to the epoxide giving a diol, a compound observed by ^{1}H and ^{13}C NMR analyses. Fig. 1 shows typical FT-IR spectra of samples taken at different time points from the reaction mixture. A growing peak at 2110 cm^{-1}, belonging to the azide group indicates that the reaction is taking place. In addition, ^{13}C NMR and DEPT-135 analyses of the reaction product (Fig. 2) show C–O peaks from the substituents at 72 and 69 ppm and a C–N_3 peak at around 53 ppm. In these conditions however, long reaction times of at least 21 h have to be used in order to achieve complete consumption of the epoxide and the reaction efficiency is rather low, ranging from only 9–13%.

Raising the reaction temperature from 30 °C to 55 °C or 70 °C slightly lowers the reaction efficiency (Table 2), but speeds up the reaction, consuming all of the epoxide within 4.5 h. Using higher concentrations of dextran give reaction efficiencies up to 30%, as more dextran hydroxyl groups become available for the etherification, but since the initial ratio of epoxide to dextran is lower, the obtained DS values are also lower. Adding more epoxide-solution gives respectively slightly higher DS values, but again, lowers the reaction efficiency. Noteworthy though that these reactions are not fully comparable, since the total volume of the reaction mixture is not equal. The epoxide-solution, which could not be concentrated or purified for safety reasons, contains isopropanol

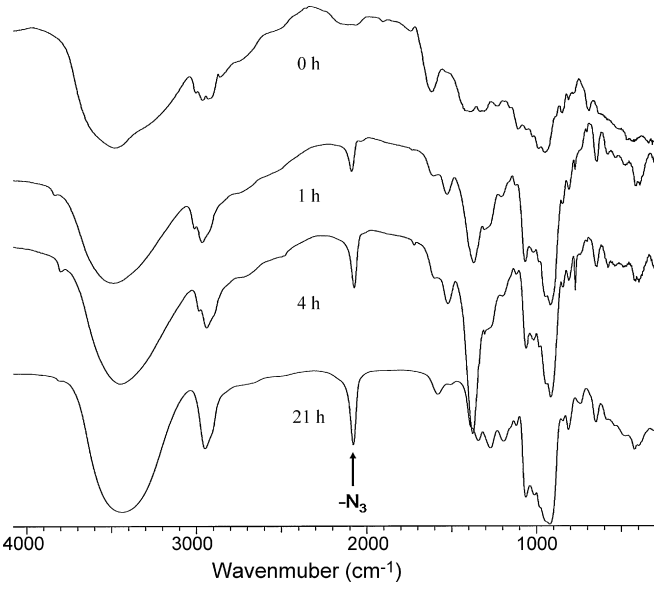

Fig. 1. FT-IR spectra of samples taken at different time points from the etherification reaction of dextran with 1-azido-2,3-epoxypropane (Table 1, entry C). A growing peak 2110 cm^{-1} indicates that azide groups are introduced to the backbone of dextran.

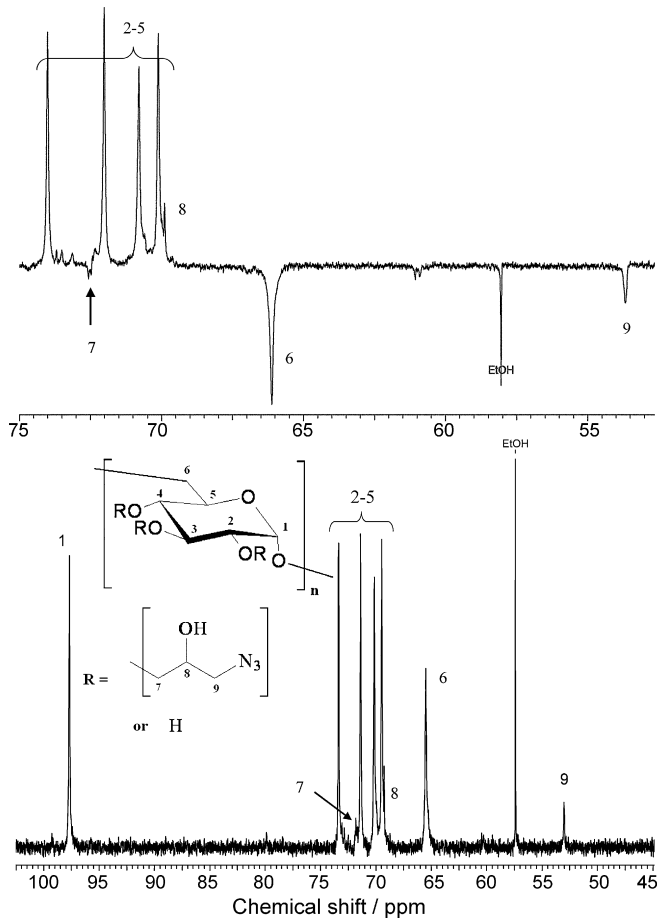

Fig. 2. DEPT-135 and ^{13}C NMR analyses (in D_2O) of the product obtained from the etherification reaction of dextran with 1-azido-2,3-epoxypropane (Table 1, entry C).

to ensure solubility of the epoxide. The solution also contains sodium acetate, NaCl and $NaNO_3$ salts from the preparation of 1-azido-2,3-epoxypropane, which may have some effect on the reaction outcome. The obtained DS values are similar to those observed with hydroxypropylation of starch in aqueous alkaline conditions (Tuschhoff, 1986). Higher azide functionalization has been reported with dextran using the tosylation approach in dry reaction conditions (Heinze, Michealis, & Hornig, 2007), however the etherification method is a simple one step synthesis and very useful when aqueous media is preferred.

3.2. Grafting of alkyne-end-functionalized PEG-MME to dextran

Two synthesized azido-dextrans of DS = 0.16 and DS = 0.08 (Table 2, entries G and O) were used to prepare dextran-*g*-poly(ethylene glycol). This was done by grafting alkyne-end-functionalized PEG-MME to the backbone of the polysaccharide utilizing copper-catalyzed azide–alkyne cycloaddition. The dextran with lower DS value (DS = 0.08) reacted quantitatively within 1 h, which can be seen from FT-IR-analysis, where the azide peak at 2110 cm^{-1} has disappeared (Fig. 3A and B). In addition, both ^1H and ^{13}C NMR spectra (Fig. 4) show peaks characteristic to both dextran and PEG, as well as signals at 8.4–8.7 ppm (^1H NMR), 144.21 and 126.37 ppm (^{13}C NMR) indicating the presence of triazole-rings formed in the CuAAC-reaction.

In the case of the dextran of higher azide content (DS = 0.16), the FT-IR spectrum of a sample taken from the reaction mixture after 1 h (Fig. 3C and D) shows that the azide functionalities are consumed in the reaction, since the peak at 2110 cm^{-1} belonging to

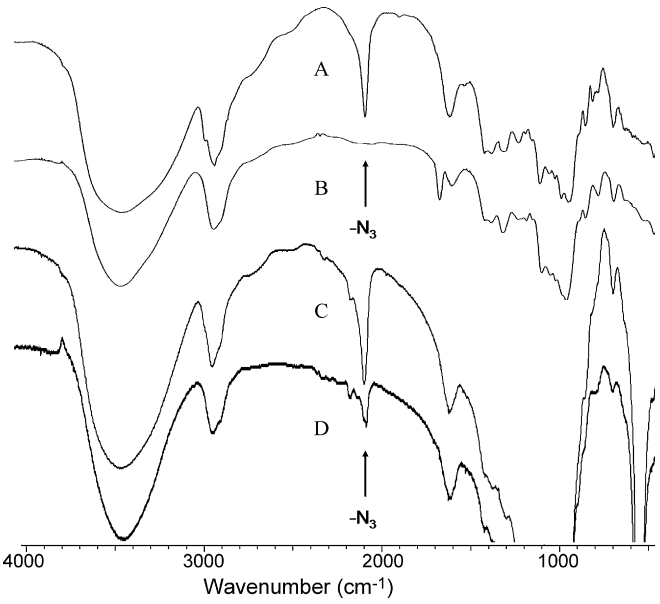

Fig. 3. FT-IR spectra of dextran containing azide groups used as starting material (A) DS = 0.08 and (C) DS = 0.16. (B) and (D) the obtained grafted products after the copper-catalyzed azide–alkyne cycloaddition reaction with alkyne-end-functionalized poly(ethylene glycol).

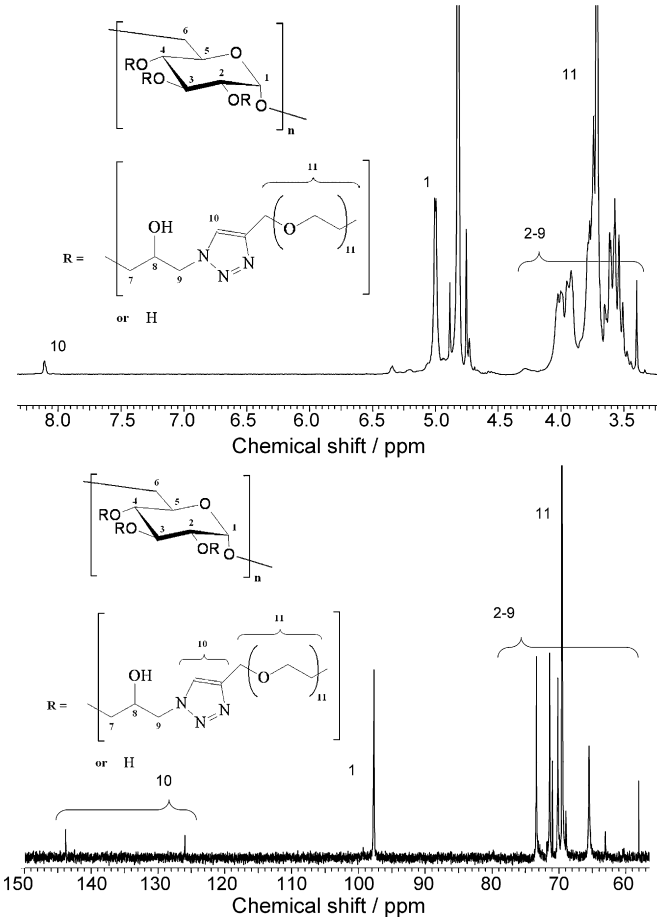

Fig. 4. ^1H and ^{13}C NMR spectra (D_2O) of the obtained dextran-*g*-poly(ethylene glycol), DS = 0.08.

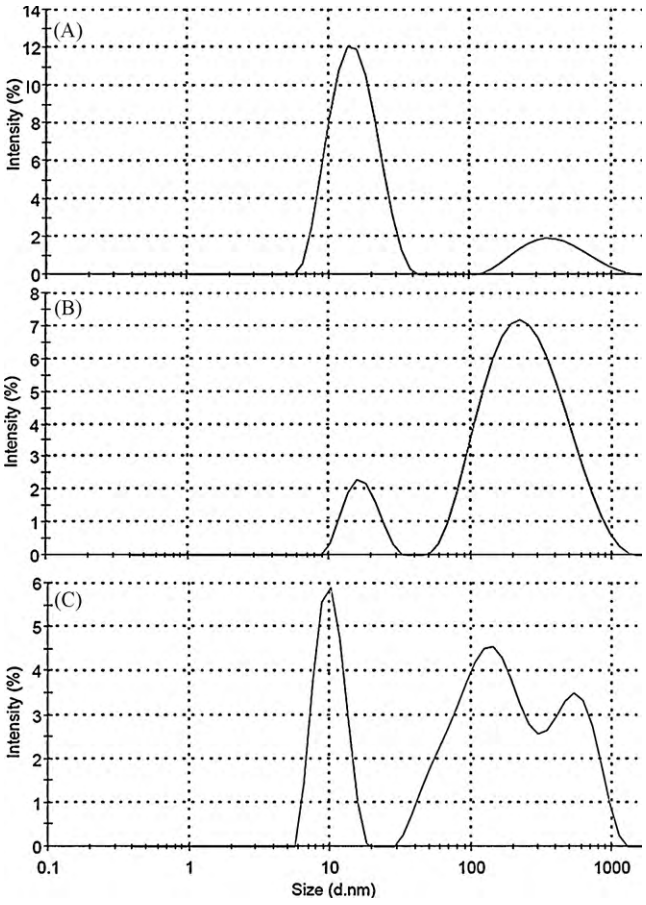

Fig. 5. Particle size distribution analyzed by dynamic light scattering of (A) dextran containing azide groups (DS = 0.16), (B) dextran-*g*-poly(ethylene glycol) after 1 h of reaction and (C) dextran-*g*-poly(ethylene glycol) after 24 h of reaction. The prolonged reaction time of 24 h may have resulted in degradation of the polymer.

azide has reduced. However, the proportional amounts of PEG and dextran units calculated from ^{1}H and ^{13}C NMR analyses correspond to a DS of 0.10, suggesting that a considerable amount (38%) of unreacted azide groups remains in the material, which can also be seen from FT-IR-analysis. Prolonging the reaction time to 24 h has no effect on the conversion, regardless of the excess of the alkyne functionalized PEG used. This may be due to steric factors, even though the molecular weight of PEG was only 500 g/mol. The prolonged reaction time of 24 h may also have resulted in degradation of the polysaccharide backbone (Lallana, Fernandez-Megia, & Riguera, 2009) and/or poly(ethylene glycol) grafts, since dynamic light scattering measurements of this sample showed reduced particle sizes (Fig. 5). The obtained dried graft copolymers were insoluble in cold water, but well soluble in warm (50 °C) water.

4. Conclusions

In this paper, the synthesis of dextran-*g*-poly(ethylene glycol) was done utilizing copper-catalyzed azide–alkyne cycloaddition (CuAAC) in aqueous media. The introduction of azide functionalities necessary for the subsequent CuAAC-reaction was done in one step by the etherification of 1-azido-2,3-epoxypropane to the backbone of dextran using NaOH-catalysis in water/isopropanol mixture. The achieved degree of substitution (DS) of azide groups was up to 0.20, when the epoxy to dextran sugar-unit ratio was 1:1. The DS values and the reaction efficiency showed to be dependent on the reaction temperature, the ratio of the reactants as well as the concentration of the polysaccharide. At low epoxide to sugar-unit ratio and high polysaccharide concentration, efficiencies up to 32% were achieved. The introduced azide functionalities could successfully be used for the grafting of alkyne-end-functionalized poly(ethylene glycol) monomethyl ether by CuAAC, yielding dextran-*g*-poly(ethylene glycol) of DS = 0.10 within 1 h of reaction. The presented combination of both traditional epoxide reactions and the more recently discovered copper-catalyzed azide–alkyne cycloaddition (CuAAC) allows aqueous reaction media to be used throughout the synthesis, making it convenient for the modification of polysaccharides for various applications.

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