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A new class of polymerizable dextrans with hydrolyzable groups: hydroxyethyl methacrylated dextran with and without oligolactate spacer

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In this paper a synthetic procedure is described towards a new class of methacrylated dextrans which are sensitive to hydrolysis. These methacrylated dextrans, which can be used for the development of biodegradable hydrogels, were obtained by grafting L-lactide onto 2-hydroxyethyl methacrylate (HEMA), followed by activation of the terminal hydroxyl group with *N,N'*-carbonyldiimidazole (CDI), yielding HEMA-lactateCI. Coupling of this compound to dextran in dimethyl sulfoxide in the presence of dimethylaminopyridine resulted in dex-lactateHEMA. In the same way, dex-HEMA was obtained by coupling of CDI-activated HEMA to dextran. The degree of substitution, as determined by ^{1}H-NMR, could be controlled by varying the molar ratio of HEMA-CI or HEMA-lactateCI to dextran. The incorporation efficiency was 60–85%. The dextran derivatives were obtained in high yield (85–90%) and characterized by NMR, FTIR and GPC. © 1997 Elsevier Science Ltd.

(Keywords: dextran; biodegradable; hydrogel)

INTRODUCTION

Hydrogels are currently under investigation as delivery systems for pharmaceutically active proteins and peptides[1–3]. Recently we reported on the release of proteins from hydrogels[4] obtained by polymerization of aqueous solutions of glycidyl methacrylate derivatized dextran (dex-MA)[5,6]. Although these gels contain methacrylate esters in their crosslinks, the hydrolysis of these groups is very slow under physiological conditions. The dex-MA hydrogels could be rendered degradable by incorporation of the enzyme dextranase, which was also an effective route to modulate the release of an entrapped protein from these matrices[7]. An alternative approach to degradable, interpenetrating networks of dextran and polymethacrylate is incorporation of hydrolytically labile spacers between the polymerized methacrylate groups and dextran. It has been reported that introduction of lactate esters induces degradability under physiological conditions in hydrogels derived from acrylated poly(ethylene glycol)-polylactate blockcopolymers[8].

In the present paper we report on the synthesis of HEMA-lactate- and HEMA derivatized dextran, a new class of polymerizable dextrans. In addition to methacrylate groups, dex-lactateHEMA contains carbonate and lactate ester groups, whereas dex-HEMA contains only additional carbonate ester groups. As shown in recent studies carried out by our group, hydrogels obtained by polymerization of these compounds degrade under physiological conditions by chemical hydrolysis of the labile ester groups present in the crosslinks[9,10]. Moreover, these hydrogels are assumed to possess a good biocompatibility since the degradation products are lactate (endogenous compound), dextran, used as plasma expander[11], and poly(2-hydroxyethyl methacrylate), a well known polymer used in many biomedical products and used for pharmaceutical applications[12].

EXPERIMENTAL

Chemicals

Dextran (from *Leuconostoc mesenteroides*, T40, M_n = 15 000, M_w = 32 500 g/mol, as determined by GPC analysis), dimethyl sulfoxide (DMSO, < 0.01% water), 2-hydroxyethyl methacrylate (HEMA, 2-hydroxyethyl methylpropenoate, less than 1% methacrylic acid (HPLC analysis)), and hydroquinone monomethyl ether (> 98% by HPLC) were obtained from Fluka Chemie AG, Buchs, Switzerland. 4-(*N,N*-dimethylamino)pyridine (DMAP, 99%) and *N,N'*-carbonyldiimidazole (CDI, 98%) were from Acros Chimica, Geel, Belgium. L-Lactide ((3S-*cis*)-3,6-dimethyl-1,4-dioxane-2,5-dione, > 99.5%) was purchased from Purac Biochem BV (Gorinchem, The Netherlands) and used without pretreatment. Stannous octoate (tin(II) bis(2-ethylhexanoate), $SnOct_2$, 95%) (Sigma Chemical Co., St. Louis, MA, USA) was used as received.

Toluene was distilled from sodium/benzophenone, and stored on molecular sieves. Tetrahydrofuran (THF) was distilled from $LiAlH_4$ immediately before use. Other

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solvents (p.a.) were obtained from Merck, Darmstadt, Germany. Dialysis tubes (cellulose, MW cut off (based on proteins) = 12 000–14 000) were purchased from Medicell International Ltd, London, UK. PD-10 columns containing Sephadex G-25 M, were supplied by Pharmacia Biotech, Uppsala, Sweden.

Characterization

NMR spectra were recorded with a Gemini 300 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA, USA). Approximately 30 mg of material was dissolved in 0.8 ml solvent. For measurements in ^2H_2O (99.8% ^2H, Merck) the ^2HOH signal at 4.8 ppm was used as the reference line, whereas in DMSO-d_6 (99.8% ^2H, Merck) containing ^2H_2O, the central DMSO line was set at 2.50 ppm. Chloroform (99.6 + % ^2H, Acros) was set at 7.26 ppm. For ^1H-NMR, a pulse length of 4.5 μs (PW_{90} 12 μs) was used with a relaxation delay of 15 s. The degree of substitution (DS; the amount of methacrylate groups per 100 dextran glucopyranose residues) of the methacrylated dextrans was calculated as $(I_a \cdot 100)/(1.04 \cdot I_{H1})$, in which I_a is the average integral of the protons (H_a) at the double bond (around 6 ppm), and I_{H1} is the integral of the anomeric proton (H_1) at δ4.95–5.1 ppm; the correction factor 1.04 is for the 4% of α − 1,3 linkages in dextran[5].

For the ^{13}C-NMR spectra, the pulse length was set at 4.5 μs (PW_{90} 12 μs), and the relaxation delay at 2 s. The $(CH_3)_3Si$ resonance (0 ppm) of sodium 2,2-dimethyl-silapentane sulfonate (DSS) was used as the reference line[13].

FTIR spectra were recorded with a Bio-Rad FTS-25 spectrometer (Bio-Rad Laboratories Inc., Cambridge, MA, USA). The dry materials were powdered, ground with dry KBr powder and pressed into pellets under vacuum. For each sample, 16 scans were recorded between 4000 and 450 cm^{-1}, with a resolution of 2 cm^{-1}.

The molecular weights and molecular weight distributions of dextran and dex-HEMA were determined by gel permeation chromatography (GPC) with a system consisting of a Waters 510 HPLC pump, and a 410 differential refractometer (Waters Associates Inc., Milford, MA, USA) with three thermostated (35°C) Shodex KW series columns (OH pack KB 800P 8 × 300 mm, precolumn; OH pack KB 802 6 mm × 50 mm, exclusion limit $4 \cdot 10^3$; OH pack KB 80M 8 mm × 300 mm, exclusion limit $2 \cdot 10^7$; Showa Denko, Tokyo, Japan). The mobile phase was degassed water containing 10 mM NaCl. The flow rate was 1.0 ml/min. The columns were calibrated with dextran standards of known molecular weight and narrow molecular weight distribution (Fluka). The chromatograms were analysed with Millennium 2010®, Version 2.0 software (Waters Associates Inc.).

Synthesis of HEMA-imidazolyl carbamate (HEMA-CI)

CDI (1.62 g; 10 mmol) was dissolved in about 15 ml anhydrous THF in a nitrogen atmosphere, and HEMA (1.30 g; 10 mmol) was added. The reaction mixture was stirred for 16 h at ambient temperature. After addition of a small amount of hydroquinone monomethyl ether (50–60 mg), the solvent was evaporated, yielding a slightly yellow liquid (yield 2.93 g). The crude product was dissolved in ethyl acetate, extracted with water to remove imidazole, unreacted CDI and HEMA, and dried on anhydrous $MgSO_4$. After filtration, hydroquinone monomethyl ether (50–60 mg) was added once more, the solvent evaporated, yielding 2.33 g of HEMA-CI (> 100%). Purity (by ^1H-NMR): 88% w/w (contains 2% (w/w) dimer, and residual solvents). ^1H-NMR ($CDCl_3$): δ8.12 (m, 1H, H_e), 7.40 (m, 1H, H_g), 7.06 (m, 1H, H_f), 6.11 (broad-s, 1H, H_a'), 5.60 (bs, 1H, H_a'), 4.64 (m, 2H, H_d), 4.48 (m, 2H, H_c), 1.92 (m, 3H, H_b) (*Figure 6*B). FTIR (KBr, in cm^{-1}): 3133 (w, $\nu_{C=C-H}$), 1765 (s, $\nu_{C=O}$ lactate and carbonate ester), 1721 (s, $\nu_{C=O}$ methacrylate ester), 1636 (m, $\nu_{C=C}$), 816 (w, $\nu_{C=C-H}$). ^{13}C-NMR ($CDCl_3$): δ 166.7 ($H_2C = C(CH_3) - C = O$), 148.3 ($O - (C = O) - Im$), 135.4 ($H_2C = C(CH_3) - C = O$), 130.6 (C_e), 126.3 (C_a), 117.0 (C_f and C_g), 65.6 (C_d), 61.5 (C_c), 18.0 (C_b).

Kinetics of HEMA-lactate formation

A mixture of L-lactide (8.64 g; 60 mmol) and HEMA (2.60 g; 20 mmol) was stirred at 110°C in a nitrogen atmosphere until the lactide was molten. Subsequently, the reaction was started by adding $SnOct_2$ (81, 203 or 405 mg; 1, 2.5 or 5 mol% with respect to HEMA, respectively), dissolved in about 0.5 ml toluene. Over a 1 h period, samples were taken periodically from the reaction mixture, cooled with liquid nitrogen and analysed with ^1H-NMR-spectroscopy. The conversion of L-lactide to HEMA-lactate was calculated from the relative intensities of the methine quartet at 5.04 (L-lactide) and 5.17 ppm (oligo-L-lactide, H_c in *Figure 4*A)[14].

Synthesis of HEMA-lactate

A mixture of L-lactide (4.32 g; 30 mmol) and HEMA (3.90 g; 30 mmol) was stirred at 110°C in a nitrogen atmosphere until the lactide was molten. Subsequently, a catalytic amount of $SnOct_2$ (121.5 mg, 1 mol% with respect to HEMA) dissolved in about 0.5 ml toluene was added. The resulting mixture was stirred for 1 h, and allowed to cool to room temperature. The reaction mixture was dissolved in THF (20 ml) and added dropwise to ice-cold water (180 ml). The formed precipitate was collected by centrifugation, dissolved in ethyl acetate (40 ml), and centrifuged to remove remaining solids. The supernatant was dried over $MgSO_4$, filtered, and concentrated under reduced pressure, yielding a viscous oil (3.74 g, 45%), consisting mainly of HEMA-lactate with 2 and 4 lactyl residues (average degree of polymerization, DP_{AV}, of 3) and residual HEMA (3 mol%).

The average length of the lactate spacer (DP_{AV}) can be increased by increasing the molar ratio of L-lactide to HEMA. HEMA (5 mmol) and varying amounts of L-lactide (10, 15, 20 or 25 mmol) were stirred at 110°C in the presence of 1 mol% (with respect to HEMA) of $SnOct_2$ in 0.5 ml toluene for 60 minutes. After cooling of the reaction mixture the products were analysed with ^1H-NMR. The workup procedure could be omitted, since at a lactide/HEMA ratio of 2 and higher, no residual HEMA was detected by ^1H-NMR (< 0.5%).

^1H-NMR ($CDCl_3$), interpretation based on HH-COSY and the literature[15,16]: δ 6.11 (s, 1H, H_a'), 5.59 (s, 1H, H_a'), 5.12–5.24 (m, H_e), 4.47–4.26 (m, 5H, H_c, H_d en H_g), 3.86 (m, H_d of residual HEMA), 2.80 (broad-s, OH), 1.94 (s, 3H, H_b), 1.39–1.59 (m, H_f and H_h), 0.90 (m, $SnOct_2$) (*Figure 4*A).

^{13}C-NMR ($CDCl_3$): δ 175.4/175.0/174.7 ($HO-CH(CH_3) - C = O$), 169.9–169.5 (cluster of five signals, $RO-CH(CH_3) - C = O$), 167.0 ($H_2C = C(CH_3) - C = O$), 135.8 ($H_2C = C(CH_3) - C = O$), 126.2 (C_a), 69.2/69.0/66.7 (C_e and C_g), 63.1 and 62.0 (C_c and C_d), 20.4 (C_h), 18.2 (C_b), 16.8/16.7 (C_f).

FTIR (KBr, in cm^{-1}): 3510 (w, ν_{O-H}), 1754 (s, $\nu_{C=O}$

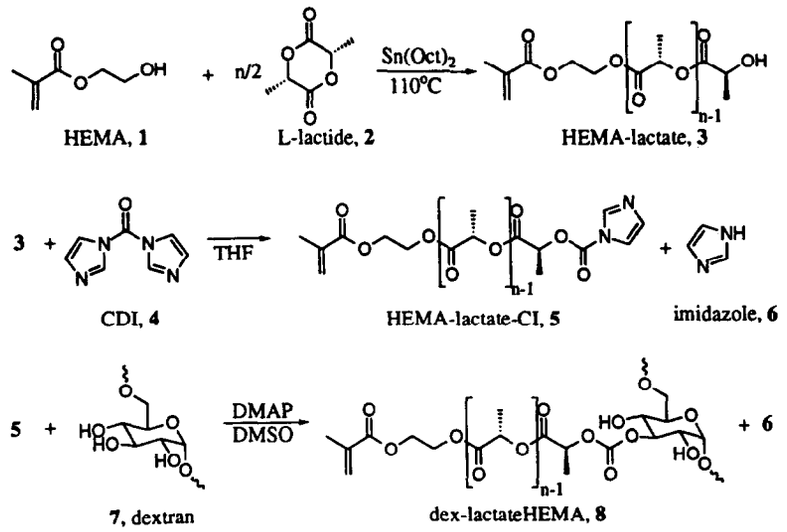

Figure 1 Reaction scheme for the synthesis of dex-lactateHEMA

lactate ester), 1723 (s, $\nu_{C=O}$ methacrylate ester), 1638 (m, $\nu_{C=C}$), 816 (w, $\nu_{C=C-H}$).

Synthesis of HEMA-lactateCI

CDI (1.76 g, 10.8 mmol) was dissolved in 100 ml THF at *ca.* 40°C in a nitrogen atmosphere and added to HEMA-lactate, synthesized as described above (3.74 g, 10.8 mmol, DP_{AV} 3). The clear solution was stirred for 16 h at room temperature. The solvent was evaporated under reduced pressure, yielding a viscous oil. The product HEMA-lactate-CI (5.50 g) was used without further purification. Composition by ^{1}H-NMR: 72% w/w HEMA-lactateCI, 8% w/w HEMA-lactate, and 20% w/w imidazole.

^{1}H-NMR ($CDCl_3$): δ 9.56 (broad-s, 1H, NH), 8.16 (m, 1H, H_i), 7.68 (m, 1H, H_m), 7.44 (m, 1H, H_l), 7.10 (s, 2H, H_p and H_q), 7.07 (m, 1H, H_k), 6.08 (m, 1H, H_a'), 5.58 (m, 1H, H_a'), 5.36 (m, 1H, H_g), 5.23–5.12 (m, H_e), 4.64 (dd, CH_2 – O-CI of residual HEMA-CI), 4.48–4.32 (m, 4H, H_c and H_d), 1.91 (m, 3H, H_b), 1.73–1.49 (m, H_f and H_h), 0.92 (m, $SnOct_2$) (*Figure 4*B).

^{13}C-NMR ($CDCl_3$): δ 169.8–168.7 (cluster of six signals, RO-CH(CH_3) – *C* = O), 166.9 (H_2C = C(CH_3) – *C* = O), 147.9 (O – (*C* = O) – Im), 135.7 (H_2C = *C*(CH_3) – C = O), 130.7 (C_i), 126.1 (C_a), 121.8 (C_m), 117.2 (C_k and C_l), 71.7/71.5 (C_g), 69.4–69.0 (cluster of six signals, C_e), 63.1/63.0 and 62.0 (C_c and C_d), 18.1 (C_b), 16.6/16.5 (C_f and C_h).

FTIR (KBr, in cm^{-1}): 3133 (m, $\nu_{C=C-H}$), 1759 (s, $\nu_{C=O}$ lactate and carbonate ester), 1722 (s, $\nu_{C=O}$ methacrylate ester), 1637 (m, $\nu_{C=C}$), 817 (w, $\nu_{C=C-H}$).

Kinetics of dex-HEMA formation

Dextran (10.0 g) was dissolved in DMSO (90 ml) in a dry, stoppered 250 ml round bottomed flask in a nitrogen atmosphere. After dissolution of DMAP (2.0 g), HEMA-CI (88% w/w pure, 3.45 g; 15.4 mmol) was added, corresponding with a molar ratio of 0.25 of HEMA-CI to glucopyranose residues in dextran. Samples (1 ml) were taken periodically from the reaction mixture and neutralized with 1.5 ml of 0.1 M HCl. The dex-HEMA was separated from unreacted HEMA-CI by elution with water over a Sephadex PD-10 column. The first 3 ml, containing the methacrylated dextran, were collected and lyophilized. The DS was determined by ^{1}H-NMR and used to calculate the incorporation efficiency of HEMA. The remaining reaction mixture was worked up by adding 2 ml of concentrated HCl to neutralize the DMAP and imidazole, transferred to a dialysis tube, and dialyzed for 2–3 days against demineralized water at 4°C. After lyophilization dex-HEMA had a DS of 20 as determined with NMR (80% incorporation).

General procedure for the synthesis of dex-HEMA and dex-lactateHEMA

Dextran (10.0 g) was dissolved in DMSO (90 ml) in a dry, stoppered 250 ml round bottom flask in a nitrogen atmosphere. After dissolution of DMAP (2.0 g), a calculated amount of HEMA-CI (purity 88%) or HEMA-lactateCI, (DP_{AV} = 3, purity 72%) was added. The solution was stirred at room temperature for 4 days, after which the reaction was stopped by adding 2 ml of concentrated HCl to neutralize DMAP and imidazole. The reaction mixture was transferred to a dialysis tube and dialyzed for 2–3 days against demineralized water at 4°C. The methacrylated dextran was lyophilized and the white fluffy product (yield 85–90%) was stored at – 20°C until use. The DS was determined by ^{1}H-NMR spectroscopy. The average incorporation of HEMA was 85% (DS < 20), and of HEMA-lactate 60% (DS < 11).

Dex-HEMA. ^{1}H-NMR (^2H_2O): δ6.18 (s, H_a''), 5.77 (s, H_a'), 5.35 (broads, H_1 of α – 1,3 branch), 5.00 (broads, 1H, H_1), 4.54 and 4.48 (broads, H_c and H_d), 4.00–3.55 (m, 6H, H_2, H_3, H_4, H_5, H_6' and H_6''), 1.95 (s, H_b) (*Figure 6*C).

^{13}C-NMR (^2H_2O): δ171.9 (H_2C = C(CH_3) – *C* = O), 158.3 and 157.5 (CH_2O-(*C* = O) – OR), 138.3 (CH_2 = *C* – CH_3), 130.0 (C_a), 100.4 (C_1), 76.1 (C_3), 74.1 (C_2), 72.9 (C_5), 72.2 (C_4), 68.3 (C_6), 69.0 and 65.7 (C_c and C_d), 20.2 (C_b).

FTIR (KBr, in cm^{-1}): 3424 (s, ν_{O-H}), 1752 (s, $\nu_{C=O}$ lactate and carbonate ester), 1718 (s, $\nu_{C=O}$ methacrylate ester), 1636 (m, $\nu_{C=C}$), 816 (w, $\nu_{C=C-H}$).

Dex-lactateHEMA. ^{1}H-NMR (12.5% ^2H_2O/DMSO-d_6): δ 5.99 (s, H_a'), 5.67 (s, H_a''), 5.14–4.95 (broad m, residual OH, H_e and H_g), 4.67 (s, 1H, H_1), 4.27 (m, H_c and H_d), 3.78 (water), 3.91–3.11 (m, 6H, H_2, H_3, H_4, H_5, H_6', H_6''), 2.50 (DMSO), 1.83 (s, H_b), 1.41 (m, H_f and H_h) (*Figure 4*C).

FTIR (KBr, in cm^{-1}): 3425 (s, ν_{O-H}), 1752 (s, $\nu_{C=O}$ lactate and carbonate ester), 1718 (shoulder of 1752, $\nu_{C=O}$ methacrylate ester), 1644 (m, $\nu_{C=C}$), 816 (w, $\nu_{C=C-H}$).

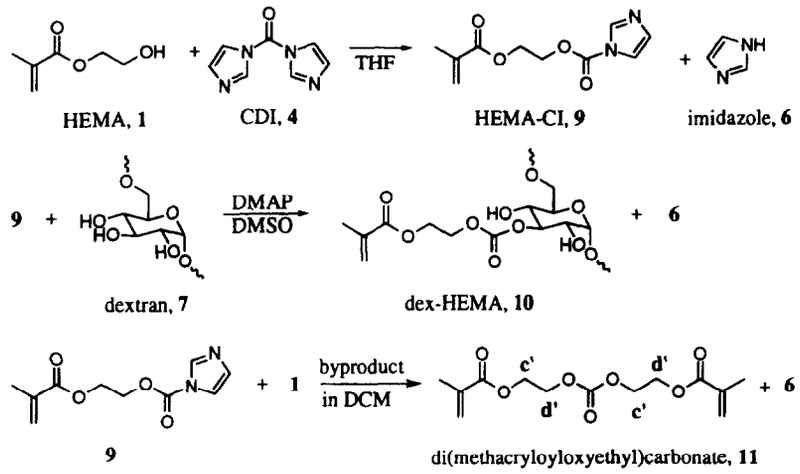

Figure 2 Reaction scheme for the synthesis of dex-HEMA

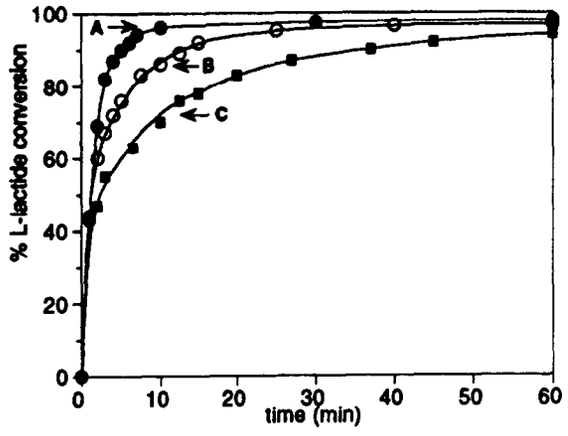

Figure 3 Conversion of L-lactide to oligolactate in the reaction with HEMA at a molar ratio of $SnOct_2$/HEMA of 0.05 (A), 0.025 (B), and 0.01(C)

RESULTS AND DISCUSSION

Synthetic approach

In the synthesis of dex-lactateHEMA three steps can be distinguished (*Figure 1*). First, L-lactide (**2**) is grafted onto HEMA (**1**), yielding HEMA-lactate (**3**). After activation with *N,N'*-carbonyldiimidazole (CDI, **4**), the resulting HEMA-lactateCI (**5**) is coupled to dextran (**7**) to yield dex-lactateHEMA (**8**).

A comparable dextran derivative without lactate spacer between the methacrylate ester and dextran was also synthesized. For this compound (*Figure 2*), HEMA was activated with CDI, and the resulting HEMA-CI (**9**) is then coupled to dextran, yielding dex-HEMA (**10**).

Synthesis of HEMA-lactate

The synthesis of HEMA-lactate has been described before[15,16]. This compound was obtained by solution polymerization of L-lactide onto HEMA in toluene, using triethyl aluminium as catalyst. This procedure, however, is sensitive to the presence of traces of water. Another catalyst, which is often used for the grafting of L-lactide onto hydroxy compounds, such as benzyl alcohol[17] or poly (ethylene glycol)[18] is stannous octoate ($SnOct_2$). We investigated whether this approach would be successful for the synthesis of HEMA-lactate. The polymerization temperature was set at 110°C to ensure melting of L-lactide and to prevent thermal polymerization of HEMA. First, a kinetic study was done to establish the optimum in the amount of $SnOct_2$ and the reaction time. In *Figure 3*, the lactide conversion is plotted as a function of time for three molar ratios of $SnOct_2$/HEMA, with a lactide/HEMA ratio of 3 (mol/mol). This figure shows that decreasing the molar ratio from 0.05 to 0.01 resulted in a lower reaction rate. However, at the lowest ratio of $SnOct_2$/HEMA investigated (0.01), the conversion of L-lactide was still almost complete (95%) in 60 min. In a separate experiment, with equimolar amounts of L-lactide and HEMA the conversion was shown to be more than 99% within an hour. Therefore, for the standard preparation of HEMA-lactate (molar ratio in the feed 1/1) the reaction time was set at 1 h, with a molar ratio of $SnOct_2$/HEMA of 0.01. The homopolymerization of L-lactide is assumed to be negligible in the presence of an alcohol under the polymerization conditions selected, since stannous alkoxide, the coordination product of $SnOct_2$ with the alcohol, is the actual initiating species[19]. After this first coordination step, the stannous alkoxide polarizes the carbonyl group in L-lactide, resulting in insertion of L-lactide between tin and the alkoxy group. These steps are repeated until all L-lactide is consumed.

When equimolar amounts of HEMA and L-lactide were used, about 15% of unreacted HEMA remained in the reaction mixture. In HEMA-lactate a secondary hydroxyl group is present which can react with lactide. This will result in a mixture of mainly HEMA-lactates with 2 and 4 lactyl residues per HEMA, and unreacted HEMA. Since contamination of HEMA-lactate with HEMA would result in a mixed dextran derivative, with and without lactate spacer, HEMA had to be removed from the product. First, removal of HEMA by extraction with water layer was tried. This resulted in the formation of quite stable emulsions, with a considerable loss of material. Therefore, precipitation from THF into cold water was used, resulting in a reduction of the amount of HEMA from 15 to 3%. A side-effect of this method was that the isolated HEMA-lactate (yield 45%) had a slightly higher average degree of polymerization of the spacer (DP_{AV},) than the DP_{av} of the raw product (3.0 *versus* 2.3, respectively). Probably, HEMA-lactate with 2 lactyl residues has a higher water solubility than HEMA-lactate with longer lactate grafts.

Other methods which were unsuccessful in reducing the amount of HEMA in the product, were carrying out the reaction in solution (THF or toluene) to better control the reaction of HEMA with L-lactide, or adding a slight excess of L-lactide (e.g. 1.1 equivalent). Separation of

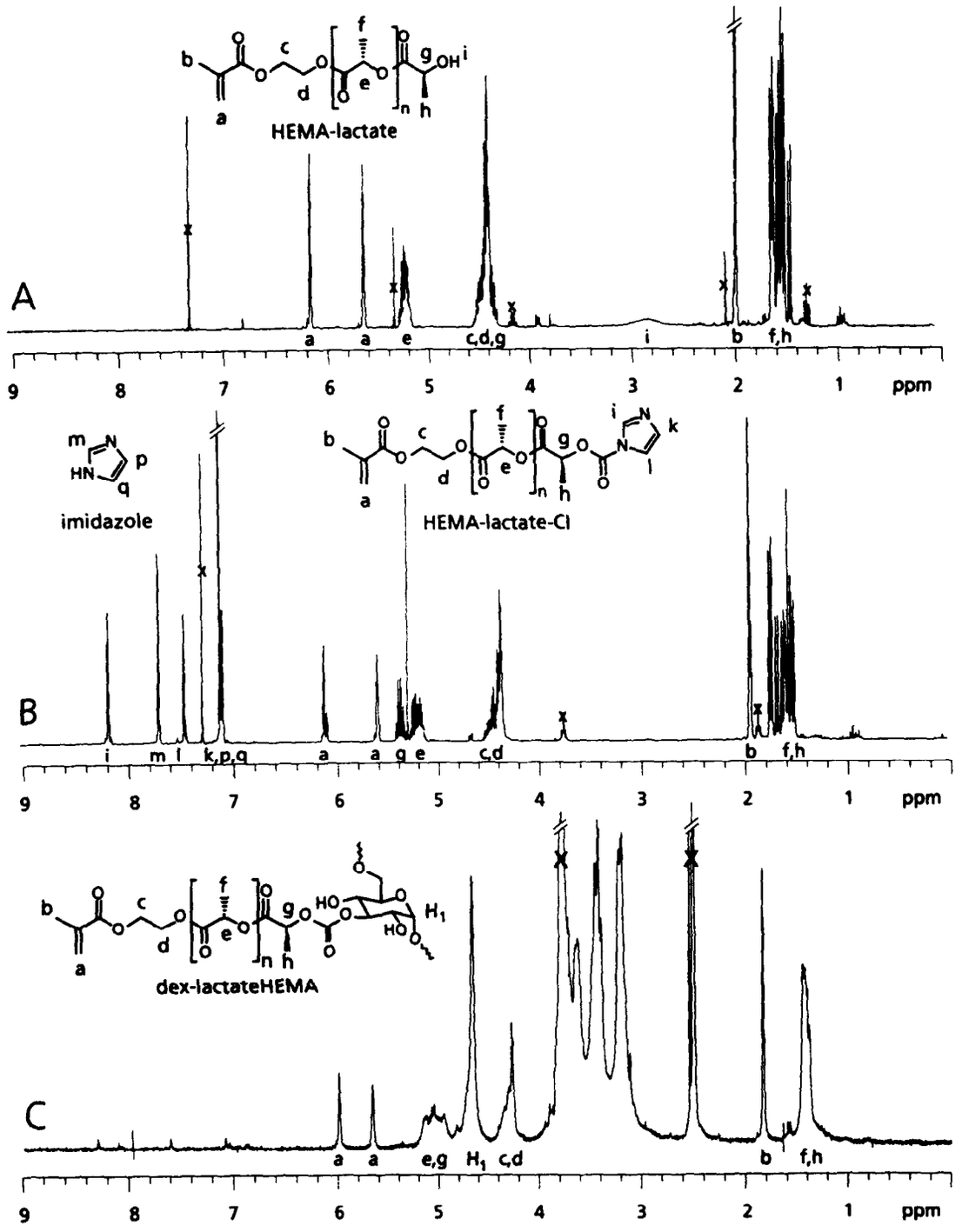

Figure 4 300 MHz ^{1}H-NMR spectra of HEMA-lactate in $CDCl_3$ (A), HEMA-lactateCl in $CDCl_3$ (B), and dex-lactateHEMA in 12.5% ^2H_2O in DMSO (C)

HEMA from HEMA-lactate by silica column chromatography with ethylacetate/hexane was successful, but this method is labourious, and has a low capacity, and is therefore not useful for preparations on a larger scale.

^{1}H-NMR analysis of HEMA-lactate (*Figure 4*A) shows, in addition to signals of the HEMA residue (H_a, H_b, H_c and H_d), also signals from the lactyl residues at 1.4–1.6 ppm (H_f and H_h) and at 5.2 ppm (H_e). H_g only shows up in the integral; this signal coincides with H_c and H_d. The spectrum shows that HEMA is indeed esterified with the lactate graft, since the chemical shift of H_d in HEMA is at 3.8 ppm (*Figure 6*A), whereas in HEMA-lactate this shift is at 4.4 ppm. Unreacted HEMA (3%) is still visible at 3.8 ppm. The DP_{AV} of the lactate graft in HEMA-lactate can be calculated from the ratio of the integrals of H_e and H_a, increased with one for the lactyl end group (the NMR signal of H_g is masked by the 2-hydroxyethyl group at 4.4 ppm).

Figure 5 shows the relationship between the molar ratio of L-lactide to HEMA in the reaction mixture and the amount of lactyl residues per HEMA molecule (which equals DP_{AV}) in the product. It demonstrates that the length of the lactate spacer can be controlled by varying the ratio of L-lactide to HEMA in the feed. At a lactide/HEMA ratio of 4 and higher, the average spacer length is slightly less than calculated from the feed ratio (*Figure 5*, dashed line). This is caused by incomplete polymerization of L-lactide within 60 min (95%). It is therefore recommended to increase the reaction time for HEMA-lactate with longer lactate grafts.

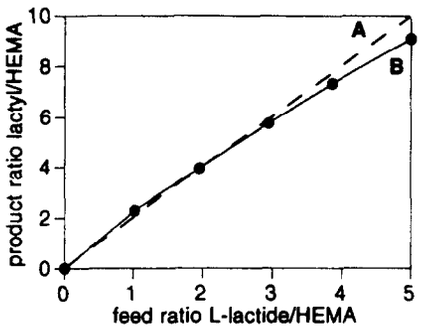

Figure 5 Relationship between the average amount of lactyl residues per HEMA in the product and the molar feed ratio of L-lactide/HEMA, calculated from the feed ratio (dashed line), and obtained from ^{1}H-NMR (solid line)

Activation of HEMA and HEMA-lactate with CDI

HEMA and HEMA-lactate, in the following referred to as HEMA(-lactate), can be coupled to a hydroxyl group of dextran via a mixed carbonate ester. This linkage can be conveniently introduced with *N,N'*-carbonyldiimidazole (CDI)[20]. First, HEMA(-lactate) reacts with an equimolar amount of CDI, yielding the imidazolyl carbamate HEMA-(lactate)CI. The activation of the hydroxyl group was established by ^{1}H-NMR.

Comparison of *Figure* 6A with 6B shows that the H_d of HEMA after reaction with CDI has shifted from 3.8 to 4.6 ppm, and that the imidazole signals have appeared at 7.0, 7.2 and 8.2 ppm. Initially, when the synthesis of HEMA-CI was carried out in dichloromethane, the product was contaminated with a considerable amount (up to 20 mol%) of

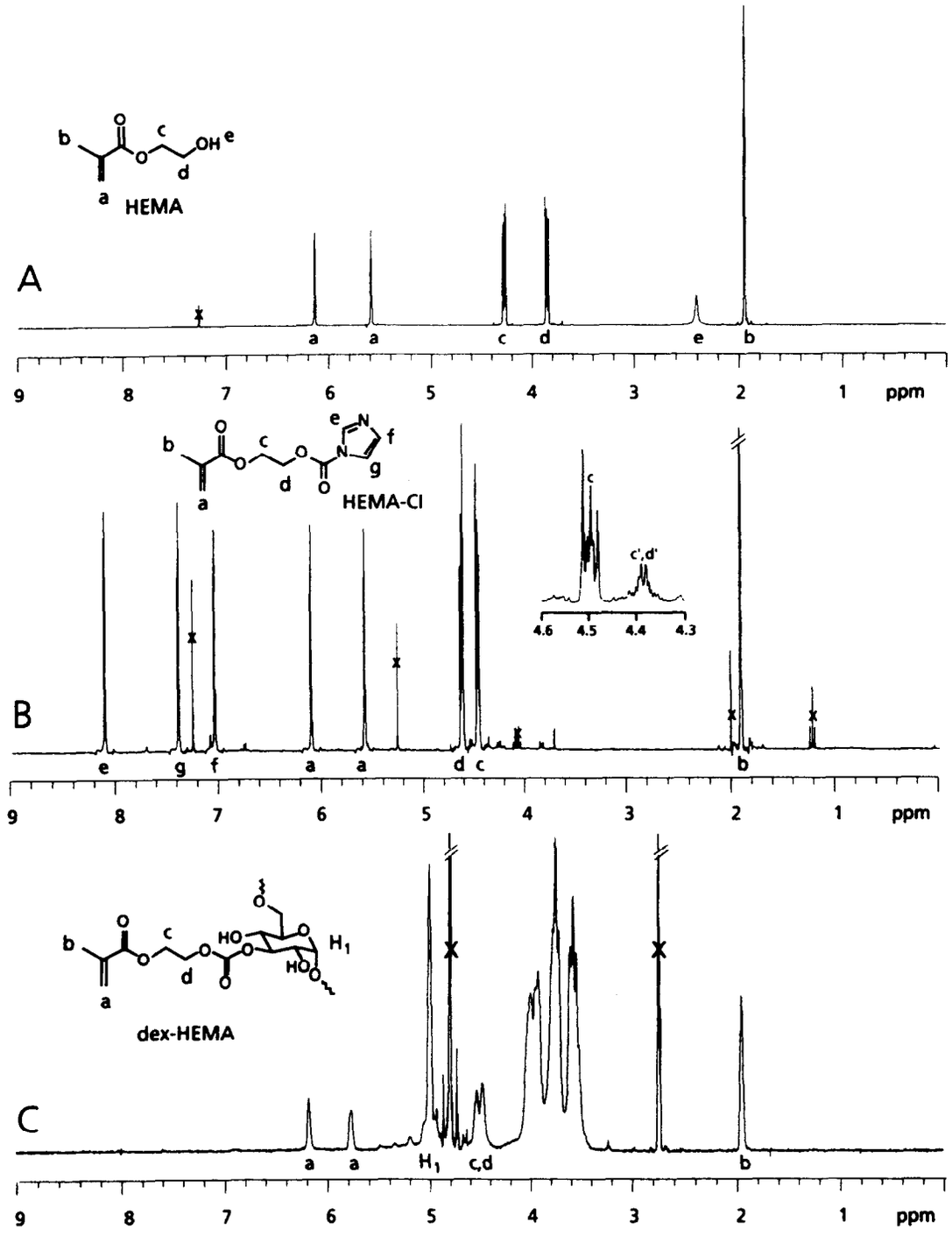

Figure 6 300 MHz ^{1}H-NMR spectra of HEMA in $CDCl_3$ (A), HEMA-CI in $CDCl_3$ (B) and dex-HEMA in ^2H_2O (C)

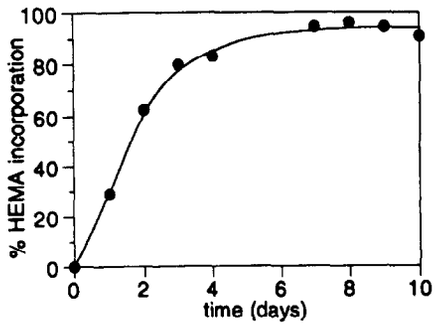

Figure 7 Incorporation of HEMA-CI in dextran as a function of time. The calculated DS from the molar feed ratio is 25

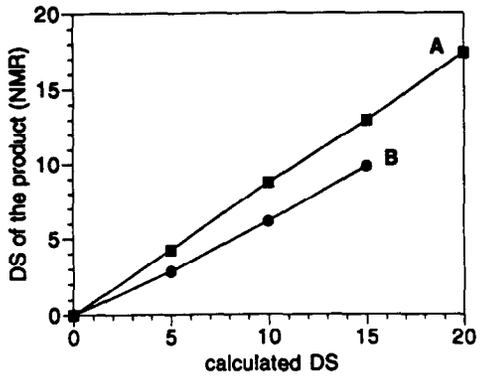

Figure 8 Relationship between the molar feed ratio of HEMA-(lactate)CI to glucopyranose residues in the reaction mixture (= calculated DS) and the DS of the product, dex-HEMA (A) and dex-lactateHEMA (B)

di(methacryloyloxyethyl) carbonate (*Figure 2*, **11**). H_c' and H_d' in this compound give a signal at 4.4 ppm (*Figure 6*B, inset). Since **11**, formed by reaction of a second molecule of HEMA with HEMA-CI, is not reactive towards dextran, care was taken to minimize this side reaction. Changing the solvent from dichloromethane to THF resulted in almost quantitative formation of HEMA-CI, with about 1 mol% of **11**. The HEMA-CI was obtained in almost quantitative yield after removal of imidazole by solvent extraction.

HEMA-lactate was also activated with CDI, yielding HEMA-lactateCI. *Figure 4*B shows the ^{1}H-NMR spectrum of HEMA-lactateCI, in which the methine proton H_g appears at 5.35 ppm, with a concomitant reduction of the integral of the peak at 4.4 ppm. From the integral ratio of H_g and H_a the extent of activation can be calculated, which is about 90%. The residual amount (about 10%) is probably present as unactivated HEMA-lactate, with its distinctive signals in the ^{1}H-NMR spectrum (*Figure 4*B) overlapping with the hydroxyethyl group at 4.4 ppm.

HEMA-lactateCI could not be purified by extraction with water, because stable emulsions were formed with several organic solvents. Since imidazole probably does not adversely affect the coupling of the HEMA-lactateCI to dextran in DMSO, as demonstrated before in the coupling reaction of glycidyl methacrylate to dextran[5], HEMA-lactateCI including the by-product imidazole, was used for the derivatization of dextran.

Coupling of HEMA-(lactate)CI to dextran

For the synthesis of dex-(lactate)HEMA, essentially the procedure for dex-MA in DMSO in the presence of DMAP was used[5]. First, the incorporation of HEMA-CI in dextran was studied as a function of time to determine the optimal reaction time. *Figure 7* shows that in 4 days the incorporation reached 85% and after that only slowly increased to 95% in 10 days. The degree of substitution did not significantly decrease with longer reaction times, which indicates that under these circumstances the incorporated spacer is not eliminated by attack of a neighbouring hydroxyl group in dextran, as observed for 4-nitrophenylcarbonate esters in dextran[21].

Figure 8 shows the relationship between the molar ratio of HEMA-CI and HEMA-lactateCI (DP_{AV} = 3) to glucopyranose residues in dextran in the reaction mixture (i.e. the calculated DS), and the DS of the products, as determined by NMR-analysis after 4 days reaction time. It is shown that the degree of substitution of dex-(lactate)HEMA can be tailored by the molar ratio of HEMA-(lactate)CI to dextran in the reaction mixture. For dex-HEMA the incorporation efficiency was 85%, whereas for dex-lactateHEMA about 60% of HEMA-lactateCI was incorporated in the product.

Although for dex-HEMA the DS could be increased to 17, attempts to synthesize dex-lactateHEMA (DP_{AV} = 3) with a DS higher than 11 were not successful. When the incorporation of HEMA-lactate in dextran reached this level, the viscosity of the reaction mixture increased and resulted in solidification, despite the presence of hydroquinone monomethyl ether as a radical inhibitor. FTIR analysis of the dialyzed and freeze-dried, water insoluble product showed that double bonds were still present*, indicating that the solidification was probably not due to chemical crosslinking of the methacrylate groups. This suggests that specific interactions between the HEMA-lactate spacers may be responsible for the solidification, since this phenomenon was not observed in dex-HEMA with a comparable DS.

GPC analysis of dex-HEMA (DS from 3 to 17) showed that the elution profile was not significantly different from dextran (results not shown), indicating that the hydrodynamic radii of dextran and dex-HEMA are equal.

*Figure 4*C and *Figure 6*C show the ^{1}H-NMR spectra of dex-lactateHEMA (DP_{AV} = 3) and dex-HEMA, respectively. Since dex-lactateHEMA is poorly soluble in water, NMR analysis was done in DMSO, with 12.5% ^2H_2O to exchange the hydroxylic protons. In addition to the lactate group at 1.41 and at 4.95–5.15 ppm in dex-lactateHEMA (*Figure 4*C), the hydroxyethyl group was visible around 4.5 ppm in both derivatives, in a 1:1 molar ratio with respect to the methacrylate ester (*Figure 4*C and *Figure 6*C). In a recent study we determined the position of the methacrylate ester at the glucopyranose ring in dex-MA with ^{13}C-NMR[6]. This method could not be applied for dex-HEMA and dex-lactateHEMA, since the DS could not be increased to a value high enough to obtain a spectrum, in which the ^{13}C signals of the substituted glucopyranose could be distinguished. However, since the reaction of HEMA-(lactate)CI with dextran also is a S_N2 substitution, we assume that the substituents are attached to the 2- and 3-hydroxyl group in the glucopyranose ring.

CONCLUSIONS

A universal method has been developed to couple hydroxyl

* The ratio of the height of the peaks at 813 cm^{-1} (double bond of methacrylate ester) and 763 cm^{-1} (dextran) is an indication for the DS of the product, as determined with ^{1}H-NMR (see [5])

containing compounds to dextran. This method is based on the activation of a hydroxyl group with CDI, followed by the coupling of the activated compound to dextran. With this procedure, both HEMA and HEMA-lactate were coupled to dextran, yielding dex-HEMA and dex-lactateHEMA, respectively. The degree of substitution can be fully controlled by the molar feed ratio of activated hydroxy compound to dextran, with 60–85% incorporation. The compounds can be polymerized in aqueous solution to form hydrogels, which hydrolyze under physiological conditions, due to the presence of hydrolytically labile crosslinks[9,10].

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