Drug Diffusion in Neutral and Ionic Hydrogels Assembled from Acetylated Galactoglucomannan

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ABSTRACT: In this study, hydrogels based on acetylated galactoglucomannan (AcGGM)—a hemicellulose present in softwood—were synthesized and examined for their properties in drug-release systems using two model substances of different molecular weight, size, and polarity (caffeine and vitasyn blue). Neutral hydrogels were produced from functionalized AcGGM using hydroxyethyl methacrylate (HEMA) coupled via carbonyldiimidazole (CDI) and a *co*-monomer in a radical-initiated polymerization. Through a second modification reaction between the HEMA-modified AcGGM (M-AcGGM-*m*ethacrylated AcGGM) and maleic anhydride, a "double-modified" AcGGM (CM-AcGGM-*c*arboxylated M-AcGGM) was successfully formed that could be cross-linked to form ionic hydrogels by the very same polymerization method. The neutral hydrogels showed drug release kinetics that could be easily regulated by changing the relative amount of the methacrylated AcGGM and its corresponding degree of methacrylation. The drug release rate and the Fickian swelling decreased with an increase in these two aforementioned parameters. The ionic hydrogels showed quicker release kinetics and higher swelling capabilities than the corresponding nonionic gels did, especially at neutral conditions. Under acidic conditions, the release speed was lowered as expected because of protonation of carboxylic functionalities. Based on the findings we conclude that these novel hemicellulose-containing hydrogels have future prospects in drug release formulations, e.g., in a later stage of development for application in oral drug administration technology. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2401–2412, 2009

Key words: hemicellulose; hydrogels; renewable resources; release; cross-linking

INTRODUCTION

Drug delivery systems utilizing hydrogels (polymeric material that swells in water but does not dissolve) based on natural components are rapidly growing in importance. Hydrogels can effectively serve as a drug vehicle in several different drug administration routes where the peroral stand out as the most important one.[1] Polymeric-based tablet cores or coatings for a controlled, delayed or sustained release formulation intended for the oral route can for example offer a protection against the acid conditions in the stomach in the same time as maintaining a steady drug concentration in the plasma for extended periods without using a repetitive administration. Colon specific drug delivery systems (CSDS) based on colon-specific degradation of polysaccharides are an advancing topic and natural non α-glucans, such as cellulose and hemicelluloses, can for example be fermented in the small intestine.[2]

Hemicelluloses are commonly defined as cell wall heterogeneous polysaccharides, which are not cellulose, and can be extracted by aqueous alkaline solutions. In softwoods, such as spruce, they consist mainly of an acetylated galactoglucomannan (AcGGM) and arabinoglucuronoxylan, representing altogether about 20–30% of the total mass. A typical segment of a spruce AcGGM, as sketched in Figure 1, consists of 0.1–1 : 1 : 4–6 galactose:glucose:mannose,[3–5] much depending on the extraction method (i.e., alkaline or neutral under hot steam or microwave treatment), having an acetyl substitution of 0.30–0.33[5,6] and an average DP below 150 (probably around 80–100).[3,7] Isolated AcGGM from wood shows excellent low-viscosity solubility in polar solvents such as water or DMSO allowing a facile accessibility for chemical modification.

Polysaccharides are generally excellent raw materials for hydrogels and an interesting feature is that

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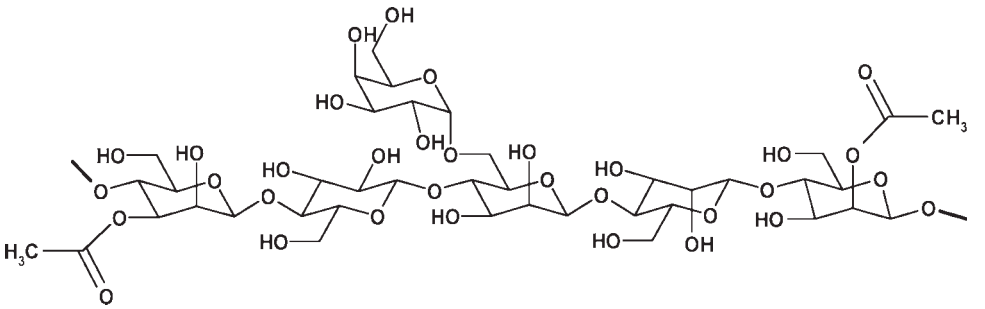


Figure 1 Fragment illustrating the structure of an acetylated galactoglucomannan hemicellulose abundantly present in softwoods.

different functionalities can easily be incorporated in the physical or chemical cross-inked network giving rise to stimuli-responsive physical properties (normally temperature-, pH-, or osmosis-controlled changes).[8] In a broader term, hydrogels also play an active and growing role as functional matrices in tissue engineering because of their versatile physicochemical properties.[9] It is always of interest to explore new polysaccharide-based hydrogels and their properties; not at least in view of that each application will require an optimal rate-control that preferably is varied by the specific matrix selection. More general information on this topic can be found in a recent published work of biomedical applications of polysaccharide hydrogels.[10]

Within our research group, it has been discovered that a galactoglucomannan hemicellulose obtained from steam-treated spruce can be utilized for obtaining high-quality hydrogel materials.[11,12] Another source that might be even more interesting in the future is the residual waste streams obtained from thermomechanical pulping (TMP) that can be ultrafiltrated and dried in an economical feasible manner.[13] Earlier we have showed that such TMP-material possesses an excellent film-forming capability combined with a very good oxygen barrier ability,[14] and can be made hydrophobic as well.[15] Furthermore, hydrogel microspheres have recently been successfully prepared and tested for their release properties using a small hydrophilic substance (caffeine) and a model protein (bovine serum albumin).[16]

In our present article, the aim was to create novel hydrogels intended for application in oral drug administration technology. For this purpose, a short-chained class of polysaccharides originating from wood was utilized, namely, acetylated galactoglucomannan (AcGGM) belonging to the hemicelluloses. Strategies to both neutral and ionic AcGGM-based hydrogels are outlined and the produced set of hydrogels were investigated for their chemical, physical, rheological, and, finally, hydrophilic drug release properties.

EXPERIMENTAL

Materials

N,N′-carbonyldiimidazole (CDI) 97% (Aldrich), 2-hydroxyethylmethacrylate (HEMA) ≥99% (Fluka), triethylamine (NEt_3) ≥99.5% (Fluka), dimethylsulfoxide (DMSO) ≥99.5% (Fluka), ethyl acetate ≥ 99% (Fluka), isopropanol ≥ 99% (Fluka), methanol ≥ 99.8% (Labscan), ammonium peroxodisulfate ≥ 98% (Fluka), sodium pyrosulfite ≥ 98% (Fluka), acetic acid, 99.7% (Aldrich), sodium sulfate 99% (Aldrich), caffeine 98% (Aldrich), vitasyn blue 98% (Aldrich) were used as received.

O-acetyl-galactoglucomannan (AcGGM) originating from spruce (*picea abies*) was obtained from thermomechanical pulping (TMP) process water. The AcGGM was purified and concentrated by ultrafiltration from about 1 wt % to 15–20 wt % followed by freeze-drying.[14] The carbohydrate composition of the AcGGM-isolate was determined to 17% glucose, 65% mannose, 15% galactose using the method of Dahlman et al.,[17] and had an average molecular weight of about 7,500 g mol^{-1} (DP ~ 40) and a polydispersity index (PDI) of ~ 1.3 as determined by size exclusion chromatography (SEC) calibrated with MALDI-TOF-MS—characterized AcGGM SEC-fractions according to the method of Jacobs and Dahlman.[18] The composition agreed fairly well with other investigations involving dissolved material from TMP.[14,19] However, Willför et al.[19] reported on substantially lower ratios of galactose moieties. Different isolation methods and their resulting products have recently been covered by Ebringerová et al.[20]

Preparation of 2-[(1-imidazolyl)formyloxy]ethyl methacrylate

2-[(1-imidazolyl)formyloxy]ethyl methacrylate (HEMA-Im) was synthesized according to a procedure reported in literature[21] with slight modifications. Nineteen milliliter (20.3 g, 156 mmol, 1 eq) 2-hydroxyethyl methacrylate (HEMA) was dissolved in 78 mL of anhydrous

$CHCl_3$. 50.67 g (312 mmol, 2 eq) of *N,N′*-carbonyldiimidazole (CDI) were added while stirring the reaction mixture at room temperature. The organic phase was neutralized after 60 min and washed with several portions of water, dried over Na_2SO_4 and finally rotary evaporated under reduced pressure. Yield: 33.2 g (97.6%). ^{1}H-NMR (500 MHz, CDCl3): δ = 8.03 ppm (s, 1H), 7.33 (s, 1H), 6.97 (s, 1H), 6.03 (m, 1H), 5.51 (m, 1H), 4.57 (m, 2H), 4.50 (m, 2H), 1.84 (m, 3H). ^{13}C-NMR (129.8 MHz, $CDCl_3$): δ = 199.59 ppm, 148.19, 136.85, 135.37, 130.48, 126.18, 116.86, 65.55, 61.42, and 17.92.

Preparation of methacrylated AcGGM (M-AcGGM) for synthesis of neutral hydrogels

A similar procedure, as Söderqvist Lindblad et al.[11] developed, was used for the production of M-AcGGM, except for that the product was precipitated in 2-propanol instead of ethyl acetate because of less stickiness. In short, 1.5 g of lyophilized AcGGM-isolate (8.6 mmol regarding the repeating unit) was dissolved in DMSO (60 mL). Two hundred and fifty microliter triethylamine (1.9 mmol, 0.22 eq) was added as catalyst using a precision glass syringe and 1.93 g of HEMA-Im (8.6 mmol, 1 eq) was added. The reaction mixture was left stirring for between 2 and 100 h (depending on wanted conversion) at 50°C under moderate stirring. The product was precipitated by pouring the reaction mixture into 300 mL of ice-cold 2-propanol and collected by centrifugation. The solid was washed with three portions of 100 mL of 2-propanol and finally dried under vacuum (pump) for about 15 h. The degree of modification, determined by ^{1}H-NMR, ranged between 2.1 and 48.3% depending on the reaction time. Yield: 85–95%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 6.04 ppm (s, 1H, vinyl C—H), 5.70 (s, 1H, vinyl C—H), 2.01 (m, acetyl groups of AcGGM), 1.87 (s, 3H, CH_3—). IR: 3390 cm^{-1} (ν polysaccharide—OH); 1737 cm^{-1} (ν carbonate C=O); 1727 cm^{-1} (ν methacrylic ester C=O); 1631 cm^{-1} (ν methacrylic C=C); 1148 cm^{-1} (ν glycosidic C—O).

Preparation of carboxylated and methacrylated AcGGM (CM-AcGGM) for synthesis of ionic hydrogels

Typical reaction conditions were 5 mmol of M-AcGGM (0.870–1.175 g, mass used was a function of the respective degree of modification regarding HEMA-Im) was dissolved in 50 mL DMSO. Triethylamine (80 μL) and 0.123–0.490 g of maleic anhydride (1.25–5 mmol, 0.25–1 eq) were added. The reaction mixture was left stirring at 50°C for 1 h. The reaction was quenched by pouring the reaction mixture into 250 mL of ice-cold 2-propanol and the solid residue was collected by centrifugation. The product was finally washed with three portions of 100 mL of 2-propanol followed by drying under vacuum (pump) for about 15 h. The degree of modification was determined by ^{1}H-NMR spectroscopy. Yield: 90–95%. ^{1}H-NMR (500 MHz, DMSO-d_6): δ = 6.38 ppm (m, 2H), 6.04 (s, 1H, vinyl C—H), 5.71 (s, 1H, vinyl C—H), 2.01 (m, acetyl groups of AcGGM), 1.87 (s, 3H, CH_3—). IR: 3370 cm^{-1} (ν polysaccharide—OH); 2881 cm^{-1} (ν aliphatic, heteroaromatic C—H); 1737 cm^{-1} (ν carbonic acid diester C=O); 1721 cm^{-1} (ν methacrylic ester C=O); 1631 cm^{-1} (ν methacrylic C=C); 1580 cm^{-1} (ν maleic C=C); 1155 cm^{-1} (ν glycosidic C—O).

Hydrogel synthesis from M-AcGGM and HEMA

Hydrogels based on AcGGM were prepared with different compositions and polymerization parameters according to the following protocol: 165/165/100 mg of M-AcGGM (corresponding to 60/51/42 mass-%) was respectively, dissolved in 1.15/1.44/1.10 mL of de-ionized H_2O (∼ 400 wt %). 2-hydroxyethylmethacrylate (HEMA) (107/157/139 μL) was then added, respectively, and the resulting mixture was thoroughly stirred. Polymerization was initiated by subsequent addition of 45/66/58 μL of each 2 wt % ammonium peroxodisulfate and 2 wt % sodium pyrosulfite as radical initiator system. The polymerizing solution was injected into cylindrical molds (Ø = 14 mm, d = 3 mm). The molds were sealed with Parafilm™ and the mixture was left polymerizing for 6 h. After the gelation was completed the spurs of the gels were cut off and the discs obtained were immersed into an excess amount of de-ionized water for 3 days to remove nonreacted material and initiator residues. The gel discs were placed on a PTFE-foil and dried at 23°C under gentle airflow and repeated turning for 24 h. FTIR: 3387 cm^{-1} (ν polysaccharide C—OH); 2881 cm^{-1} (ν aliphatic, heteroaromatic C—H); 1719 cm^{-1} (ν carbonate); 1721 cm^{-1} (ν methacrylic ester C=O); 1633 cm^{-1} (ν methacrylic C=C); 1150 cm^{-1} (ν glycosidic C—O).

Hydrogel synthesis from CM-AcGGM and HEMA

Hydrogels based on AcGGM modified with HEMA-Im and maleic anhydride were synthesized the same way as described above for hydrogels from M-AcGGM using the respective equimolar amount of the double-modified AcGGM CM-AcGGM. FTIR: 3359 cm^{-1} (ν polysaccharide and carbonic acid—OH); 2884 cm^{-1} (ν aliphatic, heteroaromatic C—H); 1713 cm^{-1} (ν C=O); 1633 cm^{-1} (ν methacrylic C=C); 1576 cm^{-1} (ν maleic C=C); 1155 cm^{-1} (ν glycosidic C—O).

Preparation of drug-loaded hydrogels for release experiments

The drug-loaded hydrogels were synthesized using a 0.23 g L^{-1} drug solution (caffeine or vitasyn blue) as polymerization medium.

Swelling and diffusion determination

The dried gels were immersed into an excess of deionized H_2O at several temperatures (23, 37 and 55°C) and their weight gain was monitored gravimetrically after different time intervals. The mass of the wet gels (m_{wet}) was determined after removing the surface water by gently dabbing the gels with filter paper. Dry weight (m_{dry}) of the gels was determined by drying the gels at 23°C on a PTFE layer for 24 h while turning them around several times. The equilibrium swelling ratio (Q_{eq}) was determined by the following equation:

$$Q_{eq} = (m_{wet,eq} - m_{dry})/m_{dry} \quad (1)$$

where $m_{wet,eq}$ is the mass of the gel in its fully swollen condition. If the swelling is studied as a function of time, the water uptake at a specific time, M_t, and the ratio M_t / M_∞ is useful:

$$M_t = (m_{wet}(t) - m_{dry}) \quad (2)$$

$$\frac{M_t}{M_\infty} = \frac{4}{\sqrt{\pi}}\sqrt{\frac{Dt}{L^2}} \quad (3)$$

where D is the diffusion coefficient, t the time and L the thickness of the dried gel. The thickness was measured by an instrument from Mitutoyo (model IDC-112B) having an accuracy of 1 μm (a pin was mounted on the bottom plate to achieve a pin-dry hydrogel-pin measurement). At least 10 points were used for each averaged value accepted for calculation. From (3), the diffusion coefficient, D, could be obtained.

By investigating the diffusion coefficient at several temperatures (23, 37, and 55°C), the activation energy, E_a, for the diffusion of water into the dry hydrogel could be obtained from the Arrhenius equation:

$$D = D_0 \exp(-E_a/\mathrm{RT}) \quad (4)$$

Rheological characterization

Viscoelastic measurements for determination of G′ (shear storage modulus) and G″ (shear loss modulus) were performed on an ARES spectrometer (TA Instruments, Waters LLC). Samples were prepared in shape of cylindrical discs Ø = 8 mm, d = 3 mm). A parallel plate geometry with a diameter of 8 mm was used in the measurements. A dynamic frequency sweep test (strain-controlled) was performed at 25°C with each sample at 5% strain within a frequency range from 500 to 0.1 Hz. The static shear modulus, G, was acquired from an uniaxial compression experiment according to the formula:

$$F/A = \mathrm{G}(\lambda - \lambda^{-2}) \quad (5)$$

where F is the compression load, A is the cross-sectional area of the fully swollen gels, and λ the compression strain (L/L_0). Having G and the polymer volume fraction, ν_2, the cross-linking density could be calculated using the relationship:

$$\mathrm{G} = \rho {\nu_2}^{1/3} \mathrm{RT} \quad (6)$$

The values of ν_2 were obtained by measuring the volume of the gels in both dried and swollen equilibrium state. Through the knowledge of the effective cross-linking density, ρ, obtained from the static mechanical and volumetric experiments, it is furthermore possible to determine the individual value for the polymer-water interaction parameter, χ, from the Flory-Rehner-equation describing the osmotic pressure in a hydrogel:

$$\pi_{tot} = \pi_{mix} + \pi_{elas} + \pi_{ion} + \pi_{elec} \quad (7)$$

where $\pi_{mix} = (\mathrm{RT}/V_{1,\ (\text{molar vol. solvent})})[\chi {\nu_2}^2 + \nu_2 + \ln(1-\nu_2)]$ and $\pi_{elas} = \mathrm{RT}\rho({\nu_2}^{1/3} - 0.5\nu_2)$.

For a nonionic hydrogel at equilibrium swelling π_{ion}, π_{elec} and $\pi_{mix} + \pi_{elas}$ is zero meaning that eq. (7) can be expressed as:

$$\chi = -[\rho V_1({\nu_2}^{1/3} - 0.5\nu_2) + \nu_2 + \ln(1-\nu_2)]\nu_2^{-2} \quad (8)$$

Spectroscopy

^{1}H-/^{13}C-NMR-spectra were recorded at 500 MHz on a Bruker DMX 500 spectrometer using Bruker software. The samples were dissolved in $CDCl_3$ or DMSO-d_6 in sample tubes 5-mm in diameter. Nondeuterated $CHCl_3$ (δ = 7.26 ppm) and DMSO (δ = 2.50 ppm) were used as standards. The degree of HEMA-Im–substitution (DS_M) was calculated by setting the relative integral of the response of the sp^2-protons into relation with the integral of the response of the known quantity of acetyl-protons.

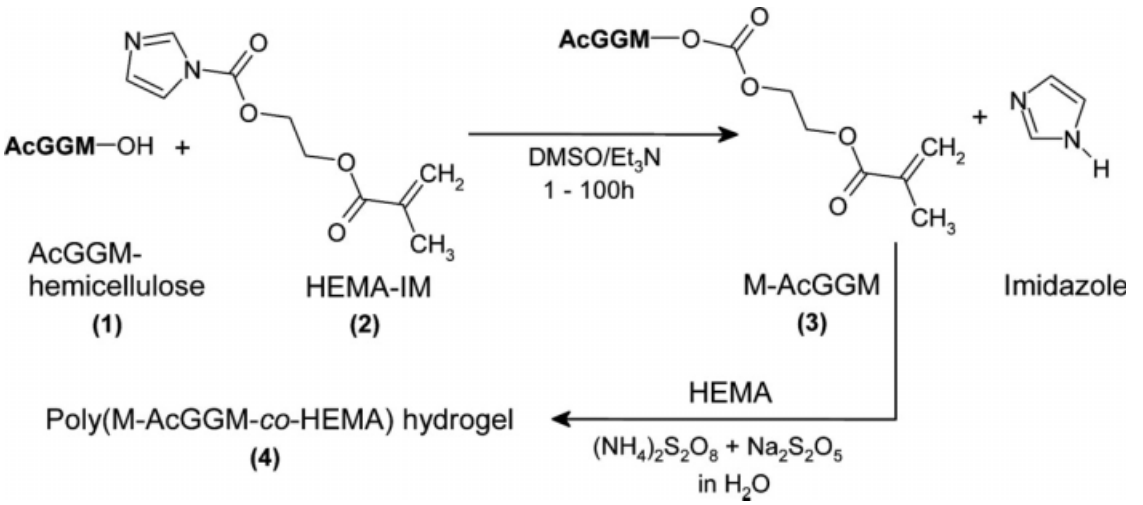
Method 1:

$$\mathrm{DS}_M = (\mathrm{I}_{sp2,HEMA}/2)/(\mathrm{I}_{acetyl}/3) \times \mathrm{DS}_{Ac} \quad (9)$$

Method 2:

$$\mathrm{DS}_M = (\mathrm{I}_{CH3,HEMA} \times \mathrm{DS}_{Ac})/(\mathrm{I}_{acetyl}) \quad (10)$$

Both methods (9) and (10) yielded about the same results and if slightly different, the average of both methods was set as the DS_M.



Scheme 1 Synthetical route applied for the preparation of poly(M-AcGGM-*co*-HEMA) hydrogels.

The degree of carboxylation (DS_C) was determined using the same method, setting the sp^2-proton response of the maleic acid into relation with the signal of the acetyl-protons.

$$DS_C = (I_{sp2,\text{maleic acid}}/2)/(I_{\text{acetyl}}/3) \times DS_{Ac} \quad (11)$$

FTIR spectra were recorded within a range from 4000 to 600 cm^{-1} on a "Perkin-Elmer Spectrum 2000"—spectrometer (Perkin–Elmer Instruments.) using "Perkin–Elmer Spectrum v3.02"–software.

Drug release analysis

Drug release analysis from the hydrogels was performed after immersing the samples in a VanKel 7010 dissolution bath (Varian, Palo Alto, CA) on a Varian Cary 50 spectrometer (Varian, Palo Alto, CA) with a 12-channel multiplexer-equipped UV fiber optic probes with 10-mm probe tips (C Technologies, Bridgewater, NJ) at 37°C and 50 rpm stirring speed using a mechanical stirrer. The raw data was analyzed using "Cary WinUV"–software (Varian, Palo Alto, CA). All measurements were performed in de-ionized water for nonionic hydrogels and for ionic hydrogels in 0.1*M* citric acid/Na_2PO_4 buffer system (pH 3), 0.1*M* phosphate buffer (pH 7) or de-ionized water.

RESULTS AND DISCUSSION

Methacrylation of acetylated galactoglucomannan (AcGGM) to M-AcGGM

Similar to a synthesis reported in literature,[11,12] methacrylic functions were attached to the AcGGM [(1) in Scheme 1] utilizing HEMA-Im [(2) in Scheme 1] in

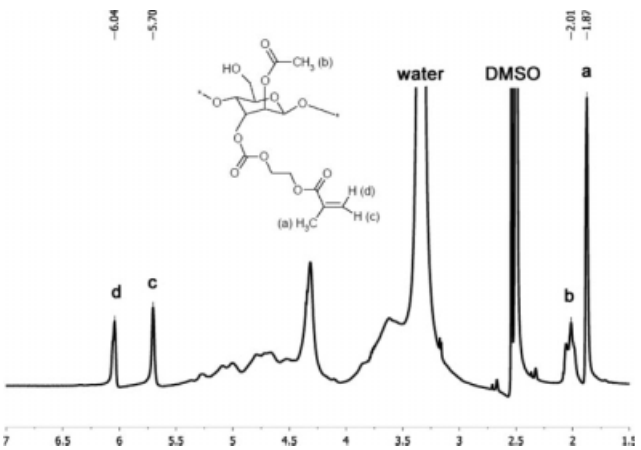


Figure 2 ^{1}H-NMR spectrum (23°C, 500 MHz, DMSO-d6) of the methacrylated AcGGM ($DS_M = 0.10$).

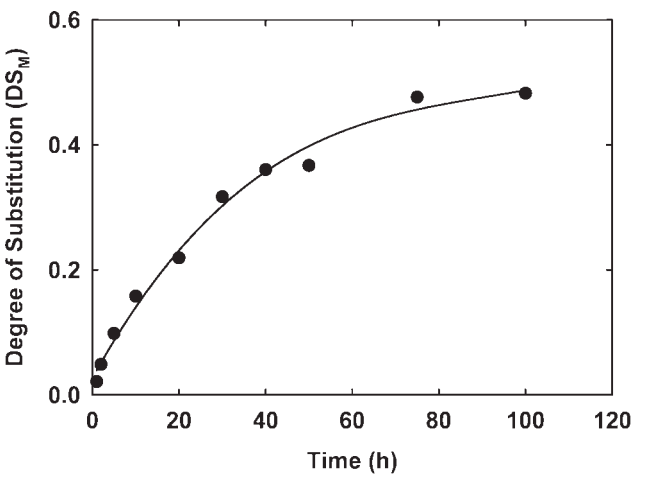


Figure 3 Observed DS for the modification of AcGGM with HEMA-Im to M-AcGGM as a function of the reaction time.

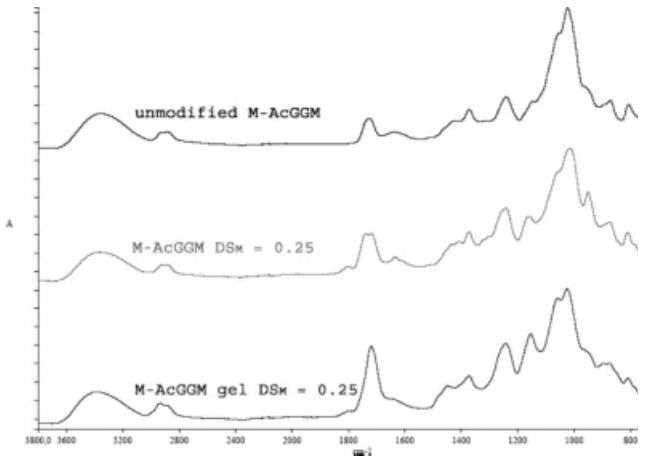


Figure 4 FTIR-spectrum (wavenumber versus absorbance) of the raw material (upper curve), the methacrylated AcGGM (DS_M = 0.25, middle curve) and of the poly (M—AcGGM-*co*-HEMA) hydrogel (DS_M = 0.25, 60 wt % content of M-AcGGM, lower curve).

DMSO as solvent at 50°C under catalysis of triethylamine. The reaction resulted in the formation of M-AcGGM containing the methacrylic function (3) as demonstrated in Scheme 1. The HEMA-Im was prepared by a reaction between HEMA and CDI (carbonyldiimidazole) in $CHCl_3$.[21] The AcGGM raw material used had its origin from thermomechanical pulping process water instead of from steam exploded wood as in the cited articles above.

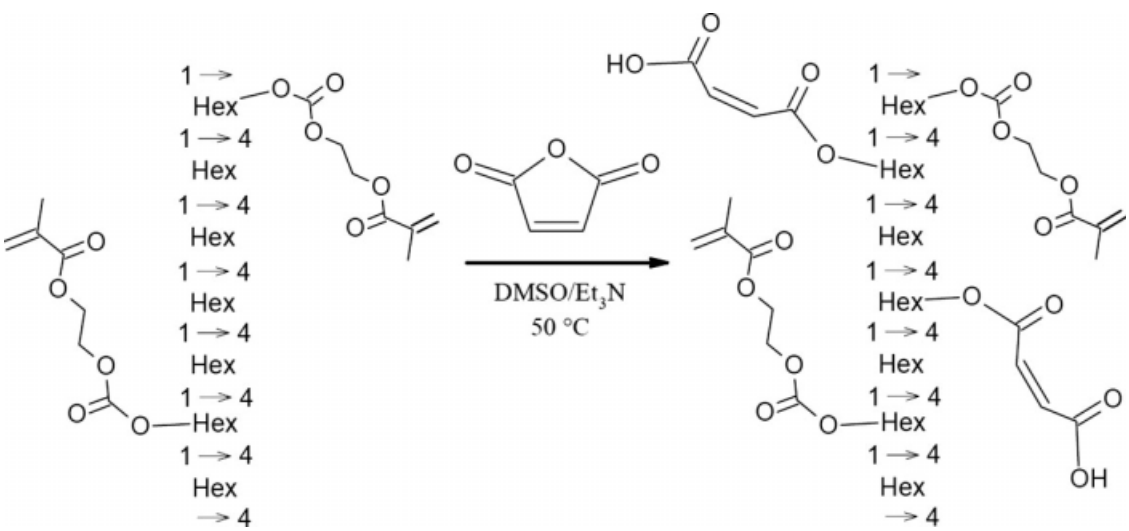
For the determination of the degree of substitution (DS) NMR spectroscopy was applied. A ^{1}H-NMR–spectrogram of an AcGGM that has reacted with HEMA-Im is shown in Figure 2. The successful modification was furthermore confirmed using FTIR (compare Fig. 4). The DS_M (the degree of *m*ethacrylic group substitution) could be calculated by setting the relative integral of the response of the sp^2-protons (6.1 and 5.7 ppm) or methyl group protons (1.9 ppm) into relation with the integral of the response of the acetyl-protons (2.0 ppm), compare eqs. (9) and (10) in Experimental. Because the degree of acetylation for AcGGM originating from spruce trees is known (DS_{Ac} = 0.30)[6] it was thus possible to quantify the DS_M.

The reaction kinetic is presented in Figure 3. As can be seen the reaction time provided fully satisfactory control over the degree of substitution. The DS_M increased with the reaction time up to a modification degree of about 0.50 after 80 h. Longer times than that did not lead to any substantial further increases in the modification degree.

Production of nonionic hydrogels from M-AcGGM and HEMA

Hydrogels were prepared by dissolving M-AcGGM [(3) in Scheme 1] together with 2-hydroxyethyl methacrylate (HEMA) in water followed by a polymerization initiated with a redox initiator system consisting of ammonium peroxodisulfate and sodium pyrosulfite. The ester of methacrylic acid and glycol, HEMA, was chosen as *co*-monomer because of its hydrophilicity on the one hand, and the excellent human tissue-like biocompatibility of poly(HEMA) hydrogels on the other.[22] To strengthen that M-AcGGM was covalently incorporated into the three-dimensional network of the hydrogel and not just acting as a nonconnected additive, a poly(M-AcGGM-*co*-HEMA) hydrogel was analyzed by means of FTIR spectroscopy together with the corresponding M-AcGGM and starting material AcGGM, see Figure 4.

A decrease of the relative absorbance was observable at the wave number of 1805, 1630, and 960 cm^{-1} from M-AcGGM to cross-linked hydrogel. These wave



Scheme 2 Reaction of a methacrylated polyhexose-model with maleic anhydride.

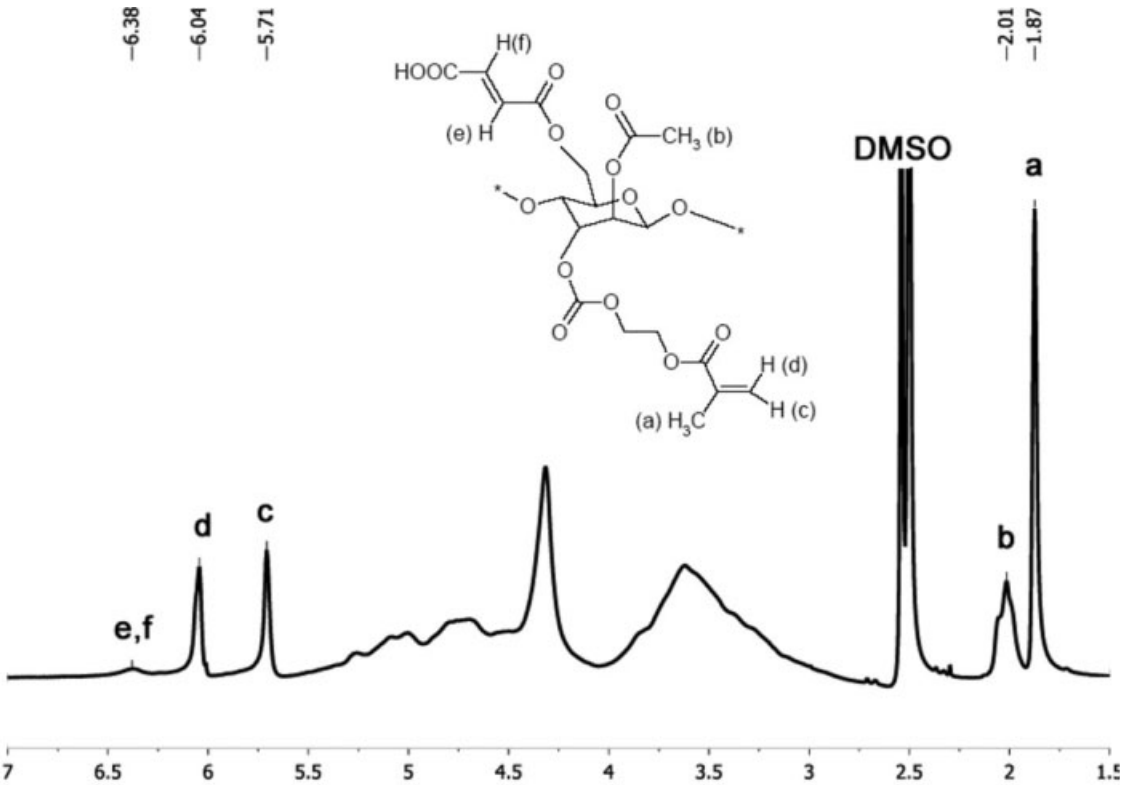


Figure 5 ^{1}H-NMR spectrum of AcGGM modified with both HEMA-Im and maleic anhydride (CMAcGGM, $DS_M = 0.48$ and $DS_C = 0.09$).

numbers can be assigned to the stretching and out-of-plane mode of a carbon–carbon double bond in the α-position to a carbonyl. Accordingly, they did not appear in the top spectra of pure AcGGM-isolate either. The disappearance of the signal changes at these wave number regions in the hydrogel indicated that a noticeable amount of the methacrylic double-bonds attached to the polysaccharide backbones had participated in a covalent incorporation of the M-AcGGM into the three-dimensional network of the hydrogel. Subsequent physical characterization strengthened these conclusions further (compare Fig. 8).

Carboxylation of M-AcGGM with maleic anhydride to CM-AcGGM and production of ionic hydrogels thereof

Although both carboxylic functionalities and double bonds are incorporated via reaction with maleic anhydride the double bonds will not be easy to polymerize and the resulting gels from maleic anhydride—modified AcGGM and HEMA will thus depend on the poly(HEMA) part rather than on a real combined network. Therefore, HEMA-Im–modified AcGGM (M-AcGGM) was altered as illustrated in Scheme 2 to a carboxylated "double"-modified derivate (CM-AcGGM–carboxylated M-AcGGM) and then polymerized the same way.

In the ^{1}H-NMR-spectrum of the new CM-AcGGM-derivate the response of the olefinic protons of the introduced maleate functionality is visible as a single broad peak at 6.4 ppm (the broadness indicates a covalent modification). An example of a NMR-spectrum of CM-AcGGM with a DS_C of 0.09 is shown in Figure 5.

The DS_C could be determined by NMR (compare Fig. 5) applying the same method as for DS_M. The

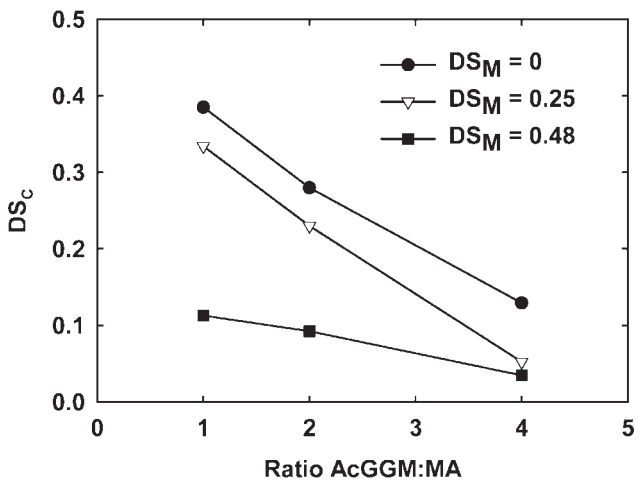


Figure 6 Observed DSC for the modification of AcGGM with different DSM as a function of molar ratio M-AcGGM to maleic anhydride (MA).

TABLE I
Parameters and Constitution of the Non-ionic M-AcGGM and Ionic CM-AcGGM Hydrogels Produced

Sample code	Wt % of /C/M-AcGGM[a]	DS_M	DS_C	Wt % of water[b]	Wt % of initiator[b]
40/10	40	0.10	–	460	0.40
40/15	40	0.15	–	460	0.40
40/22	40	0.22	–	460	0.40
60/10	60	0.10	–	420	0.40
60/15	60	0.15	–	420	0.40
60/22	60	0.22	–	420	0.40
60/10/30	60	0.10	0.31	300	0.40
60/10/50	60	0.10	0.49	300	0.40
60/10/10	60	0.10	0.10	300	0.40

[a] The remaining 40 and 60 w-% were represented by HEMA.
[b] w-% with respect to the used amount of C/M-AcGGM and HEMA together.

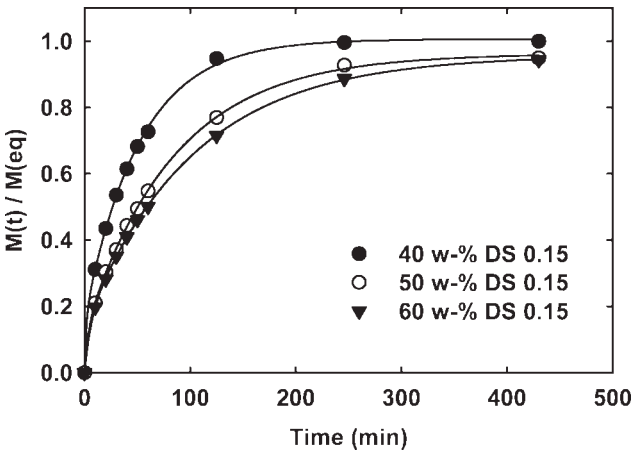


Figure 7 Plots of M_t/M_∞ versus time for hydrogels containing 40, 50, and 60 wt % M-AcGGM having a DSM of 0.15.

results showed that the DS_C was, as expected, strongly dependent on the DS_M but easy to control by means of molar ratio M-AcGGM to maleic anhydride. Figure 6 shows the correlation between molar ratio and resulting DS_C for different DS_M (0, 0.25, and 0.48).

A summary of the hydrogels synthesized and discussed in the subsequent physical and drug release examinations is presented in Table I.

Physical characterization

In Figure 7, the swelling kinetics for M-AcGGM hydrogels with different *co*-monomer amounts of HEMA is shown by plotting the fraction of water sorbed against the time.

Analyzing these data it was observed that the progress of M_t / M_∞ by time obeyed to an empirical exponential heuristic (up to a M_t / M_∞ of $\sim$ 0.6) already described by Franson and Peppas:[23]

$$M_t/M_n = kt^n \qquad (12)$$

where k stands for a characteristic constant and n for a characteristic exponent of each individual hydrogel. The exponent n describes furthermore the transportation mode of a solvent penetrating into a polymeric network and is usually found to be in a range between 0.5 and 1 where "0.5" would indicate Fickian diffusion and "1" a fully relaxation-controlled transport.[24] The values here ranged between 0.47 and 0.57, as can be seen in Table II, meaning that the swelling occurred by Fickian diffusion of the water as expected for a hydrogel. The table further reports on the equilibrium swelling, Q_{eq}, the activation energy, E_a, the static shear modulus, G, the cross-linking density, ρ, and the polymer-water interaction parameter, χ. Values for χ were all in the region of 0.5, whereas the corresponding value for a poly(HEMA) hydrogel was found to be significantly higher (0.64) suggesting a higher hydrophilicity of the poly(M-AcGGM-*co*-HEMA) hydrogels. The shear modulus, G, and the effective cross-linking density, ρ, were observed to increase as the M-AcGGM

TABLE II
Summary of the Parameters Gained from Dynamic Swelling and Static Mechanical Experiments for M-AcGGM/HEMA Hydrogels

Sample[a]	Q_{eq}[b] (g/g)	$k \times 10$[b]	n[b]	E_a [kJ/mol]	G (kPa)	ρ (μmol/cm^3)	χ
40/10	8.1	1.04	0.49	N/A[c]	0.41	N/A[c]	N/A[c]
40/15	6.8	0.90	0.49	43.26	2.83	1.65	0.53
40/22	5.4	0.57	0.57	29.49	3.89	3.40	0.55
60/10	5.1	0.56	0.56	47.21	4.69	3.86	0.53
60/15	4.2	0.52	0.52	32.78	11.16	8.25	0.53
60/22	3.4	0.50	0.50	16.01	34.89	26.41	0.53
Poly(HEMA)	1.7	0.49	0.47	–	3.01	2.07	0.64

[a] Sample codes in Table I.
[b] $Q_{eq} = (m_{wet,eq} - m_{dry})/m_{dry}$, k and n in $M_t/M_n = kt^n$ are gained from swelling experiments at 23 °C.
[c] Could not be detected due to mechanical weakness of the corresponding hydrogel.

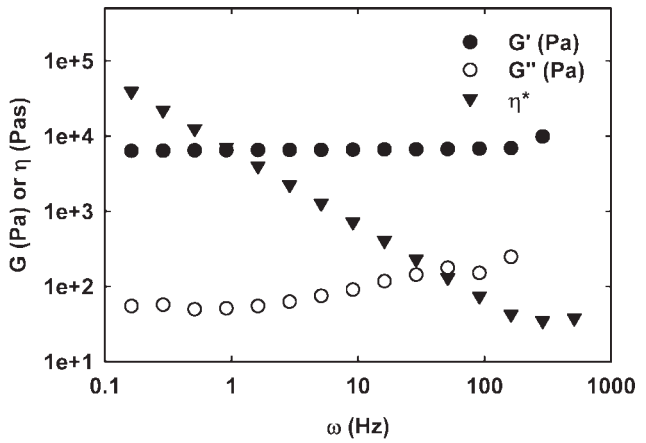


Figure 8 Shear storage and shear loss moduli and the complex viscosity as a function of frequency at 24°C for a swollen equilibrated hydrogel containing 60 wt % M-AcGGM having a DS_M of 0.15.

content and the DS_M increased. On the other hand the equilibrium swelling, Q_{eq}, was found be inversely proportional to G and ρ.

In oscillatory shear experiments a defined strain is applied periodically on the material and the measured sinusoidal stress wave can then be expressed mathematically by one in phase (G′) and one 90° out of phase (G″) shear modulus. G′ gives information on how much of the applied energy can be stored by the material in every cycle and G″ on how much energy was lost per cycle. Consequently, G″ can be also described as the shear loss and G as the shear storage modulus.

For the hydrogel investigated the G′ was much higher over a wide range of frequencies than the G.″ This is demonstrated for a hydrogel (60 wt % M-AcGGM with $DS_M = 0.15$ and 40 wt % HEMA as *co*-monomer) in Figure 8.

The fact that G′ is continuously higher than G″ demonstrates the predominately solid character according to the definition for hydrogels stated by Almdal et al.[25] Hereby, it could be proved that the poly(M-AcGGM-*co*-HEMA) hydrogels fulfill the rheological requirements on an actual hydrogel. The physical characterization moreover strongly supported that the M-AcGGM constituent participated

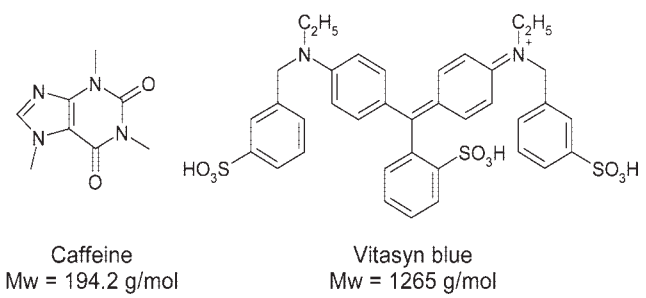


Figure 9 Molecular structures of caffeine and vitasyn blue used as model substances.

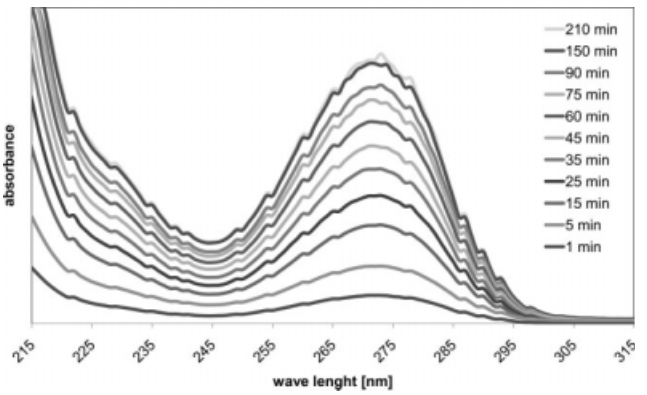


Figure 10 Time-dependent (1, 5, 10, 15, 30, 45, 60, 75, 90, 150, and 210 min with increasing absorption) UV/Vis spectra of a release experiment from an initially dry hydrogel loaded with caffeine during polymerization.

in the cross-linked network. The static shear modulus, G, was also measured and was found to vary between 0.4 and 35 kPa (compare Table II). A greater ratio of M-AcGGM and a higher DS_M of the same resulted in higher strength of the formed hydrogel and reversibly a lower swelling capability. A pure poly(HEMA) gel showed both a comparably low swelling degree and shear modulus (3 kPa), and was hence generally weaker compared with when modified polysaccharides were incorporated.

Drug release

In the drug release experiments two model substances were used, namely caffeine and vitasyn blue, and their chemical structures are shown in Figure 9.

From the UV-absorptions, as seen in Figure 10, the total amount of substance released could be calculated via the extinction coefficient.

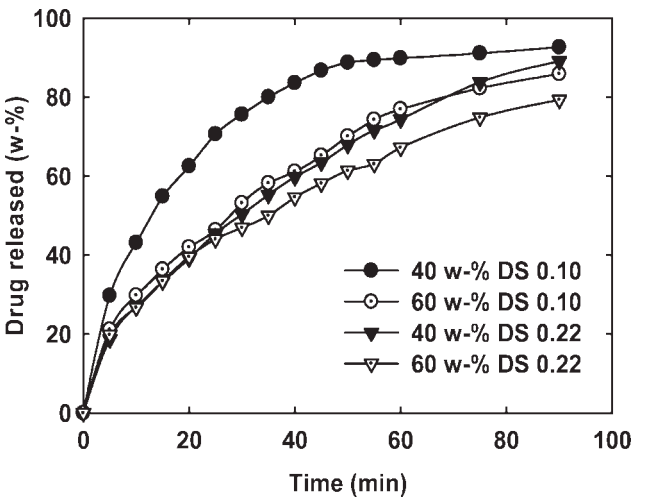


Figure 11 Caffeine release (in 900 mL H_2O, 37°C, 50 rpm stirring) from initially dry hydrogels loaded during polymerization.

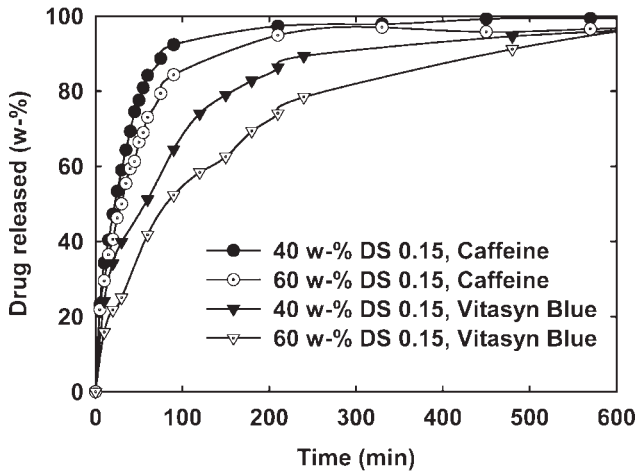


Figure 12 Caffeine and vitasyn blue releases (in 900 mL H_2O, 37°C, 50 rpm stirring) from initially dry hydrogels loaded during polymerization.

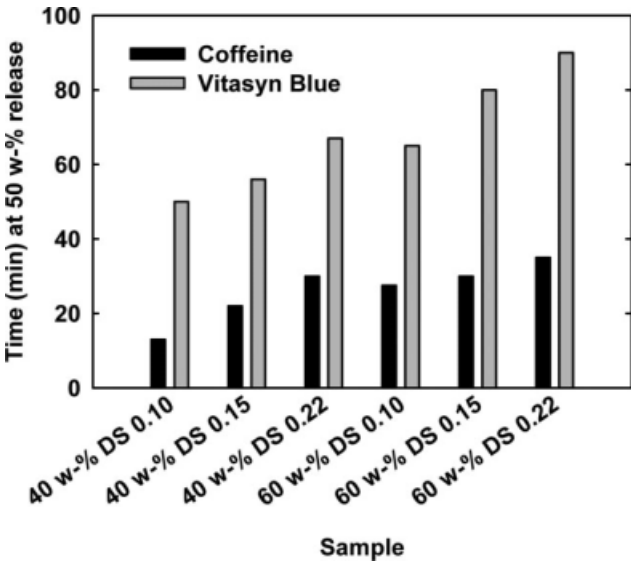


Figure 13 Time for 50 wt % release of caffeine and vitasyn blue (in 900 mL H_2O, 37°C, 50 rpm stirring) from initially dry hydrogels loaded during polymerization.

The percentage release was determined by dividing the mass of the released drug at a certain time by the total mass released. The percentage release of caffeine as a function of time for the hydrogels investigated is shown in Figure 11. The interpretation of the results is that the release is slower when the cross-linking density is higher and the poly(HEMA) chains are shorter.

The effects of the amount and the molecular structure of the substrate on the release kinetics are shown in Figure 12 where the release of caffeine and vitasyn blue is compared. As expected, the properties as molecular weight or polarity of the released species itself have a strong influence on the release kinetics; e.g., the larger vitasyn blue molecule resulted in slower release speed compared with caffeine. Furthermore, the amount of M-AcGGM had a greater impact on the release speed of the larger vitasyn blue molecule in comparison with the faster caffeine release. This is reasonable, because a larger molecule will interact with the matrix for a longer period of time. It can be observed that the release of caffeine from the manufactured discs is slower than that from microspheres earlier investigated.[16] This is simply explained by the shorter diffusional path due to the smaller size of the spheres.

The times needed for 50 wt % of the drugs to be released were determined in Figure 13. The same trend regarding the M-AcGGM content and its modification degree was observed for both model compounds and the hydrogel consisting of 60 wt % M-AcGGM with a DS_M of 0.22 and 40 wt % HEMA resulted in the slowest release (35 min for caffeine and 90 min for vitasyn blue at 50% released drug amount, see Table III). A further increase in the M-AcGGM content and its modification degree (DS_M) is expected to continue to prolong the release time. The gross (mg total) and relative (mg drug/mg hydrogel) releases together with the swelling at the drug release test temperature is given in Table III. As can be interpreted the release correlated with the swelling ability—a higher swelling resulted in a faster release.

Drug release experiments using caffeine were performed for the poly(CM-AcGGM-*co*-HEMA) hydrogels as well. The release was tested both in neutral and acidic conditions. In Figure 14, it is shown that the release was faster at pH 7.0 in comparison to at

TABLE III
Time for Total and 50 w-% Drug Release at 37°C from Initially Dry Hydrogels Loaded During Polymerization Equilibrium Swelling is Included

	Gross release (mg)		Relative release (mg/mg)		50 wt % release (min)		
Sample	Caffeine	V. Blue	Caffeine	V. Blue	Caffeine	V. Blue	$Q_{eq,\ 37°C}$
40/10	8.3	5.2	0.125	0.147	13	50	7.42
40/15	10.2	6.3	0.119	0.122	22	56	6.07
40/22	9.5	7.0	0.109	0.066	30	45	4.21
60/10	9.3	7.0	0.085	0.127	27	65	5.97
60/15	8.6	5.8	0.079	0.067	30	80	4.60
60/22	8.5	4.3	0.076	0.060	35	90	4.08

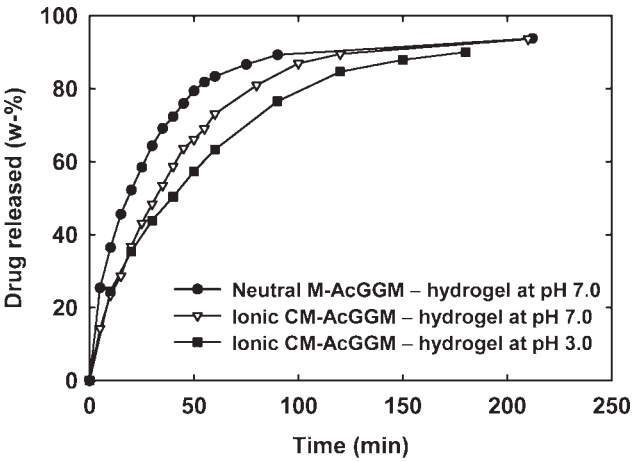


Figure 14 Caffeine release (in 900 mL H_2O, 37°C, 50 rpm stirring) from initially dry neutral (DS_M 0.10) and ionic hydrogels (DS_M 0.10, DSC 0.31) loaded during polymerization. The hydrogels were all produced with 50 wt % HEMA as comonomer.

pH 3.0. This is due to a dissociation of the carboxylic functions which leads to a swelling of the matrix due to electrostatic repulsion. If the ionic strength is raised a masking of the charges will occur leading to a less pronounced swelling. This effect explains the release results obtained from the buffered neutral conditions which gave a slower release in comparison to the corresponding nonbuffered conditions.

The 50 wt % release times are shown in Figure 15 and include a corresponding nonionic gel for the sake of comparison. The fastest caffeine release was found for the nonionic hydrogel and with increased maleic acid incorporation the release rates were lowered, even at neutral conditions. The difference in release times between neutral and acidic conditions were quite low showing a time difference of about 20% at the 50 wt % release level. Nevertheless, it could be concluded that it was possible to exert an influence of the release behavior of an ionic poly (CM-AcGGM-*co*-HEMA) hydrogel by variation of the pH.

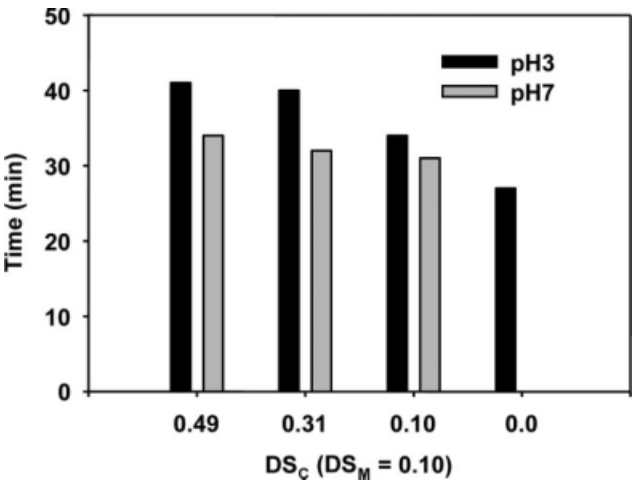


Figure 15 Time required to release 50 wt % of the caffeine loaded from the ionic hydrogels bearing a $DS_M = 0.10$ and three different DS_C in neutral and acidic conditions. For comparison, the value for a similar gel with $DS_M = 0.10$ and $DS_C = 0.0$ is also given (neutral conditions). The hydrogels were all produced with 50 wt % HEMA as comonomer.

CONCLUSIONS

Neutral and ionic hydrogels based on HEMA-Im–modified AcGGM (M-AcGGM) and maleic anhydride modified M-AcGGM (CM-AcGGM) were studied in view of their chemical, physical and drug release properties. The *co*-monomer used was HEMA. Longer release times of two differently sized model compounds were observed for higher degrees of substitution and polysaccharide mass fractions whereas the water uptake decreased with an increase in these parameters. In the case of the neutral hydrogels half of the total drug release (50 wt % release) was observed to occur between 13 and 35 min for caffeine and 50 to 90 min for vitasyn blue. The majority of the caffeine (80 wt %) was released between 40 and 120 min, whereas for vitasyn blue the corresponding figures were between 125 and 250 min. When maleic anhydride was added to the M-AcGGM producing a "double-modified" hemicellulose, ionic poly(CM-AcGGM-*co*-HEMA) hydrogels could be achieved. The release of caffeine was found to be slower in these hydrogels, especially at acidic conditions because of the pH-responsitivity obtained through the introduced carboxylic functionalities. To conclude, a new representative with most promising properties in the growing group of modified natural polymers in medical technology has been designed and preliminary evaluated in this work.

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References

1. Peppas, N. A.; Bures, P.; Leobandung, W. Ichikawa, H. E. J Pharm Biopharm 2000, 50, 27.
2. Vandamme, T. F.; Lenourry, A.; Charrueau, C.; Chaumeil, J.-C. Carbohydr Polym 2002, 48, 219.
3. Timell, T. E. Wood Sci Techn 1967, 1, 45.
4. Capek, P.; Kubackova, M.; Alfoldi, J.; Bilisics, L.; Liskova, D.; Kakoniova, D. Carbohydr Res 2000, 329, 635.
5. Capek, P.; Alfoldi, J.; Liskova, D. Carbohydr Res 2002, 337, 1033.
6. Lundqvist, J.; Teleman, A.; Junel, L.; Zacchi, G.; Dahlman, O.; Tjerneld, F.; Stålbrand, H. Carbohydr Polym 2002, 48, 29.

7. Lundqvist, J.; Jacobs, A.; Palm, M.; Zacchi, G.; Dahlman, O.; Stålbrand, H. Carbohydr Polym 2003, 51, 203.
8. Kikuchi, A.; Okano, T. Adv Drug Deliv Rev 2002, 54, 53.
9. Hoffman, A. S. Adv Drug Deliv Rev 2002, 54, 3.
10. Söderqvist Lindblad, M.; Sjöberg, J; Albertsson, A.-C.; Hartman, J. In Chemicals, Materials, and Energy from Biomass; ACS Symposium Series; Argyropoulos, D. S., Eds.; ACS: Washington, D.C., 2008; Vol. 954, ISBN: 0841239819.
11. Söderqvist Lindblad, M.; Ranucci, E.; Albertsson, A.-C. Macromol Rapid Comm 2001, 22, 962.
12. Söderqvist Lindblad, M.; Albertsson, A.-C., Ranucci, E.; Laus M.; Giani, E. Biomacromolecules 2005, 6, 684.
13. Persson, T.; Nordin, A.-K.; Zacchi, G. Jönsson, A.-S. Appl Biochem Biotechnol 2007, 136-140, 741.
14. Hartman, J.; Albertsson, A.-C.; Söderqvist Lindblad, M.; Sjöberg, J. J Appl Polym Sci 2006, 100, 2985.
15. Hartman, J. Albertsson, A.-C. Sjöberg, J. Biomacromolecules 2006, 7, 1983.
16. Edlund, U.; Albertsson, A.-C.; J Bioactive Compatible Polym 2008, 23, 171.
17. Dahlman, O.; Jacobs, A.; Liljenberg, A.; Olsson, A. I. J Chromatogr A 2000, 891, 157.
18. Jacobs, A.; Dahlman, O. Biomacromolecules 2001, 2, 894.
19. Willför, S.; Sjöholm, R.; Laine, C.; Roslund, M.; Hemming, J.; Holmbom, B. Carbohydr Polym 2003, 52, 175.
20. Ebringerová, A.; Hromádková, Z.; Heinze, T. Adv Polym Sci 2005, 186, 1.
21. Ranucci, E.; Spagnoli, G.; Ferruti, P; Macromol Rapid Comm 1999, 20, 1.
22. Kabra, B.; Gehrke, S. H.; Hwang, S. T. J Appl Polym Sci 1991, 42, 2409.
23. Franson, N. M.; Peppas, N. A. J Polym Sci Phys Ed 1986, 24, 2409.
24. Korsmeyer, R. W.; Meerwall, E. W.; Peppas, N. A. J Polym Sci Phys Ed 1986, 24, 409.
25. Almdal, K.; Dyre, J.; Hvidt, S.; Kramer, O. Polym Gels Netw 1993, 1, 5.