Journal of Applied Polymer Science

Hydrogen-Bonded Supramolecular Polymers as Self-Healing Hydrogels: Effect of a Bulky Adamantyl Substituent in the Ureido-Pyrimidinone Monomer

Traian V. Chirila,[1,2,3,4] Hui Hui Lee,[1,3] Mathieu Oddon,[1,5] Marko M. L. Nieuwenhuizen,[6] Idriss Blakey,[3,7] Timothy M. Nicholson[8]

[1]Queensland Eye Institute, South Brisbane, Queensland 4101, Australia
[2]Queensland University of Technology, Faculty of Science and Engineering, Brisbane, Queensland 4001, Australia
[3]The University of Queensland, Australian Institute for Bioengineering and Nanotechnology (AIBN), St Lucia, Queensland 4072, Australia
[4]The University of Queensland, Faculty of Health Sciences, Herston, Queensland 4006, Australia
[5]École Supérieure d'Ingénieurs de Luminy (ESIL), Polytech Marseille, Aix-Marseille Université, 13288 Marseille, Cedex 09, France
[6]Institute for Complex Molecular Systems and Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, 5600 M B Eindhoven, The Netherlands
[7]Centre for Advanced Imaging (CAI), The University of Queensland, St Lucia, Queensland 4072, Australia
[8]School of Chemical Engineering, The University of Queensland, St Lucia, Queensland 4072, Australia
Correspondence to: T. V. Chirila (E-mail: traian.chirila@qei.org.au)

ABSTRACT: In an attempt to generate supramolecular assemblies able to function as self-healing hydrogels, a novel ureido-pyrimidinone (UPy) monomer, 2-(*N*′-methacryloyloxyethylureido)-6-(1-adamantyl)-4[1*H*]-pyrimidinone, was synthesized and then copolymerized with *N,N*-dimethylacrylamide at four different feed compositions, using a solution of lithium chloride in *N,N*-dimethylacetamide as the polymerization medium. The assembling process in the resulting copolymers is based on crosslinking through the reversible quadruple hydrogen bonding between side-chain UPy modules. The adamantyl substituent was introduced in order to create a "hydrophobic pocket" that may protect the hydrogen bonds against the disruptive effect of water molecules. Upon hydration to equilibrium, all copolymers generated typical hydrogels when their concentration in the hydrated system was at least 15%. The small-deformation rheometry showed that all hydrated copolymers were hydrogels that maintained a solid-like behavior, and that their extrusion through a syringe needle did not affect significantly this behavior, suggesting a self-healing capacity in these materials. An application as injectable substitutes for the eye's vitreous humor was proposed. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39932.

KEYWORDS: biomaterials; copolymers; gels; rheology; supramolecular structures

Received 8 May 2013; accepted 3 September 2013
DOI: 10.1002/app.39932

INTRODUCTION

The strategies to induce the ability for self-repair in damaged materials have become an established field of research. Taking the formation of cracks and the ensuing fracture as a damage model, there are two paradigms along which this subdiscipline of materials science has been progressing.[1] The "damage prevention" paradigm is based on generating microstructures that are able to oppose the onset and/or the progress of damage. To this end, conventional techniques (welding, patching, or addition of new material) have been employed to repair visible or detectable damage. A drawback is that as soon as the damage has occurred, it will propagate into the bulk of material where remains undetectable, no longer responds to external intervention and, upon further exposure to load, will lead ultimately to catastrophic failure.

In biological systems any damage triggers a healing response, which is mediated through physiological processes leading to remodeling and regeneration of the damaged tissue. Despite its complexity, the natural self-healing process may serve as inspiration for designing a similar process in artificial systems.[2–5]

Additional Supporting Information may be found in the online version of this article.

The paradigm of "damage management"[1] is based on the concept that if the damage is opposed by an independent and spontaneous process leading to its removal or repair the damage will no longer be permanent. This concept defines the scope of the self-healing materials research.

A number of informative reviews[5–11] are available on remendable and self-healing polymers. Formation of supramolecular polymer networks via noncovalent interactions constitutes one of the systems leading to self-healing polymers.[5,8,9] The self-healing ability is an inherent consequence of the reversibility of noncovalent bonds. To this aim, the multiple-point hydrogen-bonding motifs have been widely used[12–28] to generate supramolecular networks through a self-assembling process. In the absence of water, the self-healing ability of hydrogen-bonded systems can manifest, for instance, upon heating, when the hydrogen bonds dissociate and the system is no longer supramolecular. As the system becomes fluid at this stage, any pre-existing damage is obliterated. Upon cooling down to the ambient temperature, the hydrogen bonds are reformed and the system becomes again a supramolecular solid, with no "memory" of the previous damage.

We are currently investigating supramolecular hydrogels as self-healing materials. While some of these hydrogels can be responsive to temperature or to pH, our interest is in those hydrogels where the only external action needed to accomplish the self-healing process would consist of bringing the fractured ends of the hydrogel into mutual contact, when the hydrogen bonds are expected to be restored. A process that does not require external stimuli is commonly called "autonomic" self-healing. While the presence of water is both unavoidable and necessary, as it defines the state of hydrogel, the self-healing can occur in a hydrated polymer if the competition by water molecules is prevented or reduced. Indeed, the hydrogen bonds are strongest in low-polarity solvents, while in the presence of protic solvents (water, alcohols), they can be weakened and eventually disrupted due to association and/or exchange events that involve the hydrogen atoms participating in the initial hydrogen bond. The molecules containing oxygen atoms (strong acceptors for hydrogen) and reactive hydrogen atoms (able to participate in proton exchange) are well-known destabilizers for the hydrogen bonds. The effect of water can be diminished by using multiple hydrogen-bonding motifs characterized by a high dimerization affinity,[29] and may additionally be reduced by shielding the hydrogen bonds with alkyl substituents, thus creating protective "pockets" (or "microdomains").[20] This approach, in which the hydrophobic interactions may also play a role, was effective in low molecular weight gelators (LMWGs),[30–42] and also in short supramolecular polymers soluble in both water and organic solvents.[43]

Our interest in a self-healing hydrogel has been triggered by the need for an artificial substitute for the eye's vitreous body (humor). Such a material is mainly required in the treatment of the vitreoretinal pathological conditions, where it has to reinstate or enhance the tamponade against a detached retina. A large number of materials have been proposed and evaluated,[44–46] but the progress was frustrated due to an insuperable problem: the only surgically acceptable procedure to insert a material into the vitreous cavity is the injection through small-gauge needles. When a gel in the syringe's barrel (large diameter) is injected, it undergoes mainly stretching flow while still in the large cylinder, but when is extruded through the needle (very small diameter) it is subjected to considerable hydrodynamic stress generating strong shear and extensional flow components and leading to gel's fragmentation when a critical value is exceeded. We have shown[47,48] that a fragmented gel cannot assure the necessary tamponade effect in the vitreous cavity, and also triggers an intensive phagocytic activity resulting in vacuolization and further fragmentation of the gel particles, leading ultimately to the opacification of the whole material. Using a self-healing hydrogel will not prevent its fragmentation caused by injection, but it is expected that the particles will not persist long enough to be phagocytized as individual entities, as they will assemble again through hydrogen bonding and regenerate either a continuous gel or a viscoelastic fluid.

To this aim, we are developing self-healing hydrogels as vitreous substitutes using the quadruple hydrogen-bonding motif introduced by Meijer's group,[29,49–56] which is based on 6-substituted 2-ureido-4[1*H*]-pyrimidinone (henceforward UPy) [Figure 1(a)]. The homodimerization by self-complementary hydrogen bonding of the *p*-quinonoid tautomers of UPy results in an AADD-DDAA array (where A and D denote hydrogen acceptor and donor, respectively), as shown in Figure 1(b), which can lead to the strong self-assembling of the "monomers" containing this motif to form supramolecular polymers. In our endeavour, we opted for a "side-chain approach," where the main chain is a covalent copolymer of a hydrogel-generating acrylic monomer and a methacrylate-functionalized UPy derivative. The pendant UPy modules will dimerize via hydrogen bonding to generate noncovalent crosslinks between two covalent main chains, leading to supramolecular networks.

In the present study, we describe a system where the main chain has resulted by copolymerizing *N*,*N*′-dimethylacrylamide with a methacrylate-functionalized UPy in which the 6-substituent is adamantyl [Figure 1(c)]. The focus of our study is on the preparation and properties of this novel monomer and its capacity to generate hydrophobic pockets that may be effective in assuring the survival of hydrogen bonds in aqueous media, which should be consequently reflected in the rheological behavior of the resulting hydrogel.

EXPERIMENTAL

All reagents and solvents, the instruments used in this study, as well as the synthetic procedures, yields and analysis of compounds **2**, **3** and the monomer 2-(*N*′-methacryloyloxyethylureido)-6-(1-adamantyl)-4[1*H*]-pyrimidinone (**4**) are detailed in the Supporting Information; 2,7-*bis*(dodecanoylamino)-1,8-naphthyridine (compound **5** in Scheme 1)[57] was a gift from the Eindhoven University of Technology, The Netherlands. Details of the copolymerization of monomer **4**, size exclusion chromatography and rheology of copolymers, and evaluation of monomer content in copolymers are also given in the Supporting Information. Throughout this report, all percentage concentrations or composition ratios were expressed by weight (w/w), unless otherwise specified.

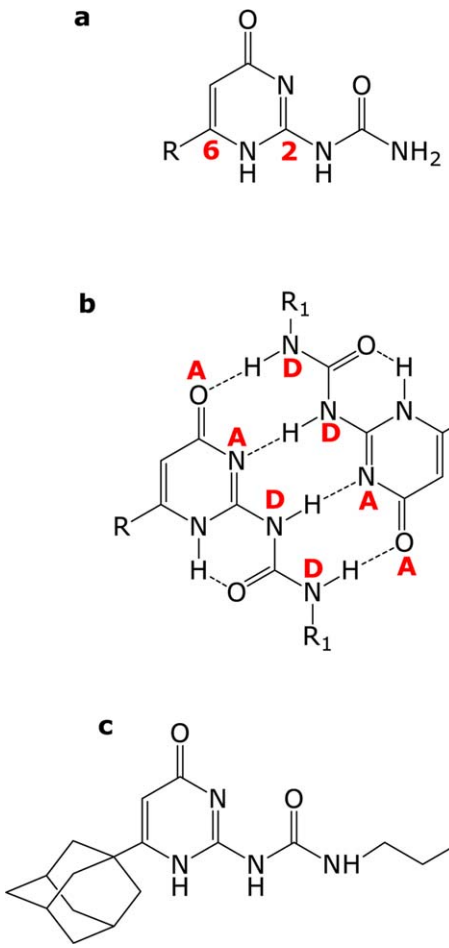
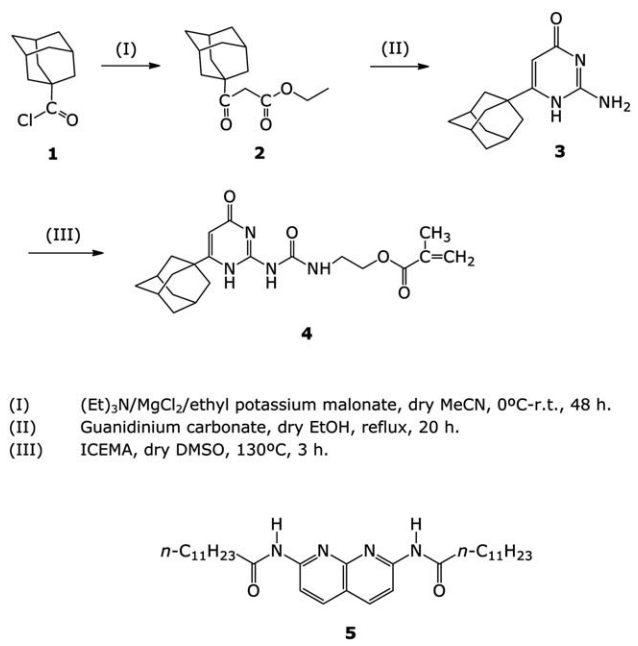


Figure 1. Structures of 6-substituted 2-ureido-4[1*H*]-pyrimidinones (a), their hydrogen-bonded homodimer array (b), and 2-(*N*′-methacryloyloxyethylureido)-6-(1-adamantyl)-4[1*H*]-pyrimidinone (c). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]



Scheme 1. Synthetic routes and compounds.

Copolymerization of Monomer 4 with N,N-Dimethylacrylamide (DMAA)

Compound **4** displayed very low solubility in the common organic solvents. In *N,N*′-dimethylacetamide (DMAc), our solvent of choice for performing the polymerization, the solubility of monomer **4** was lower than 0.2 mg in 1 mL solvent. To enhance this value, we dissolved lithium chloride, LiCl, in DMAc and used this solution as a polymerization solvent. The lithium salt was purified according to a published method.[58] The maximum solubilization effect was achieved with solutions of 6% of LiCl in DMAc. Four copolymers were synthesized at four different feed concentrations (1.5, 3.0, 4.5 and 5.9%) of the comonomer **4** in DMAc-LiCl.

RESULTS AND DISCUSSION

Synthesis of Compound 3

Our route to the adamantyl-substituted pyrimidinone **3** was different from that reported by another group,[59,60] where the reaction was carried out in the presence of stoichiometric amounts of potassium *tert*-butoxide. Because this procedure required an additional step (evaporation of ethanol under reduced pressure) and considering that the reported yield (41%)[60] was not particularly high, we decided to follow the classic method, notwithstanding the existence of other reports on the alkoxide method.[61–66] The method we used in this study was reported for the first time in 1886 by Behrend,[67,68] and employed subsequently with satisfactory yields.[50,69–75] The global yield of the reaction can be improved by assuring propitious conditions for enhanced recovery of the precipitate, e. g. low temperatures during filtration and washings, and additional recovery of precipitate from the first-run filtrates by re-precipitation with large amounts of water. However, the latter is a rather tedious operation and perhaps not justified in view of the benefit.

Synthesis of Monomer 4

To our knowledge, the compound **4** has not been previously reported. A number of articles report the synthesis of a methacrylate-functionalized UPy (the 6-methyl substituted derivative) starting from isocyanatoethyl methacrylate (ICEMA).[76,77] The two reported procedures differed from each other although both originated in the same laboratory. In the first version,[76] ICEMA was added at once over a solution of pyrimidinone in dimethyl sulfoxide (DMSO) heated to 170°C, a method used subsequently by others.[78] In the second version,[77] all reactants were added at the beginning in DMSO and heated to 150°C.

In our procedure, ICEMA was added dropwise while maintaining a relatively constant temperature in the reaction mixture, which was significantly lower than those in the mentioned methods. Thus, we maintained an excess of amine reactant (the pyrimidinone) over the isocyanate reactant, such assuring a rapid consumption of the latter, and also avoiding uncontrolled exothermal temperature bursts. Phenothiazine (PTZ) (200 ppm) was added to ICEMA as a polymerization inhibitor, while hexamethylenetetramine (HMTA) (20 ppm) was added

to the reaction mixture with the dual purpose of preventing coloration (due to PTZ) and acting as a catalyst for the reaction between amine and isocyanate groups. The complete removal of DMSO traces from the final product proved to be inordinately challenging and required washing with large volumes of methanol and acetone followed by prolonged drying in vacuum.

Solubilization of Monomer 4

A known problem with the UPy derivatives is their low solubility in common solvents, which is important for their polymerization. Taking advantage that monomer **4** is an amide, we used a solubilization system which is also used for the dissolution of peptides. The salt-mediated solubilization of amino acids and polypeptides was first recognized long ago by Pfeiffer.[79–81] Some alkaline and alkaline earth metal salts have solubilizing effects when present as solutes in the dissolving medium, and lithium halides proved to be the most powerful solubilizing agents not only for peptides and proteins, but also for other biopolymers (e. g. polysaccharides), synthetic polymers and nonpolymeric organic compounds.[82–89] Studies by IR, UV and ^{1}H NMR spectroscopies[90–93] indicated that Li^+ ions interact with the amide groups by binding preferentially to the carbonyl oxygen, and $Li\cdots(O)_4$ tetrahedral complexation was suggested. The complexation can disrupt the interchain associations in polymers, such enhancing their solubility. In particular, the LiCl-DMAc solvent system has shown a high solubilizing potential for compounds containing the amide group. This is a situation where LiCl causes the enhancement of solubilization in a solvent which contains the same functional group as the compound to be solubilized.[94]

In our experiments, we used a solution of LiCl as the polymerization medium. At a concentration of 6% LiCl in DMAc, the solubility of monomer **4** increased from <0.2 mg/mL to >50 mg/mL.

Supramolecular Polymerization

The supramolecular hydrogel investigated here belongs to the class of "closed supramolecular assemblies" as defined by Ciferri.[95–97] In such systems, two covalent polymer chains, or a chain and other covalent molecules (e. g. in the case of host-guest assemblies), are associated through noncovalent interactions through side-chain modules, in this instance hydrogen-bonding motifs. Such systems were alternatively called "crosslinked networks of side-chain polymers,"[13] "side-chain hydrogen-bonded polymers,"[15] or "supramolecular polymer networks,"[98] and they do exist in nature (e. g. DNA).[99] The synthetic closed supramolecular assemblies have been pioneered by Kato and Fréchet.[100–102] More recently, such systems have been studied as LMWGs[103] or as crosslinked supramolecular polymers.[98,104] In the "open supramolecular assemblies," also termed "linear supramolecular polymers,"[98] which have been studied to much greater extent, the addition of successive repeating covalent chains proceeds by intermolecular noncovalent end-to-end aggregation in a manner reminiscent of the molecular covalent polymerization.

It should be mentioned that the 6-methyl analogue of the monomer **4** has been used to create supramolecular polymers based either on side-chain crosslinking[76–78,105,106] or on end-to-end association.[107–109] The systems described in these reports were not hydrogels, and their rheological behavior was investigated in melts or non-aqueous solutions with the aim to improve their processing.

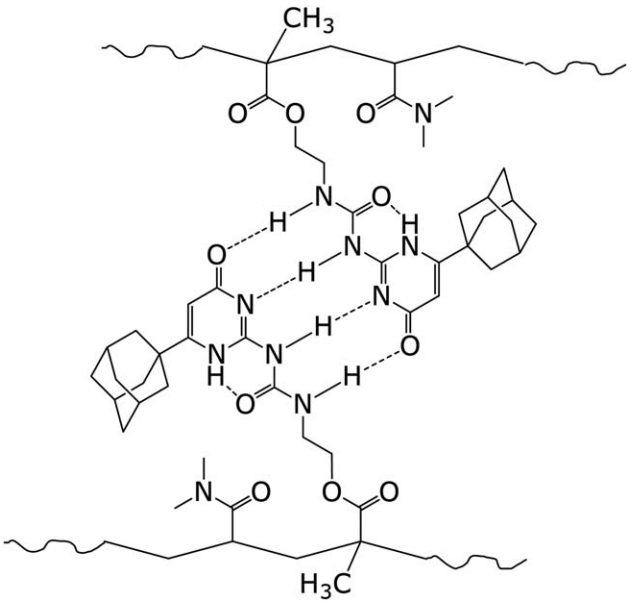
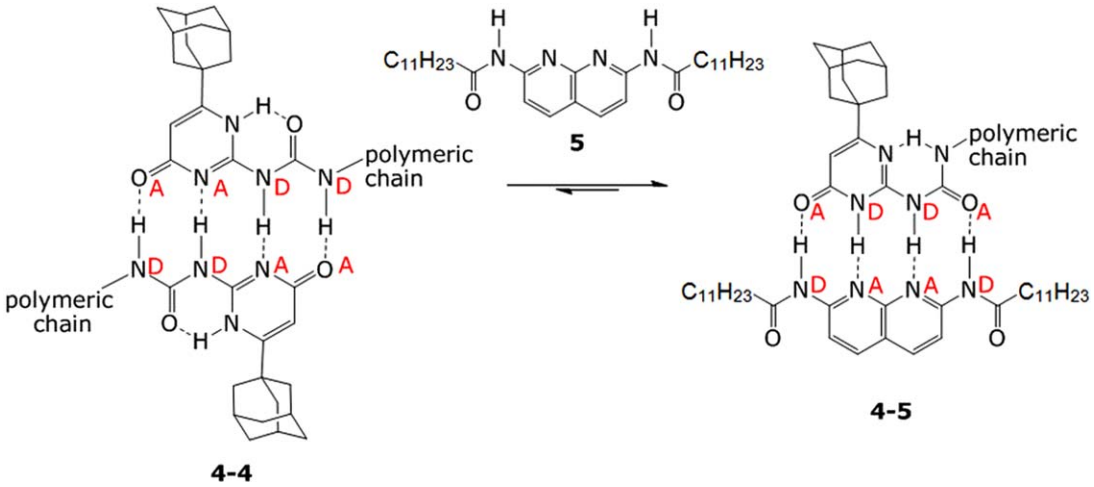


Figure 2. Structure of the supramolecular crosslinking unit in poly{*N*,*N*′-dimethylacrylamide-*co*-[2-(*N*′-methacryloyloxyethylureido)-6-(1-adamantyl)−4[1*H*]-pyrimidinone]}.

In the copolymer of *N*,*N*′-dimethylacrylamide (DMAA) with monomer **4**, discussed here, the intermolecular (and intramolecular) hydrogen bonding is the driving force for the self-assembling process that leads to a supramolecular hydrogel (Figure 2). The hydrogen bond is remarkable through its specificity (i.e., it forms only when an H atom bound to an electronegative atom interacts at the same time with another electronegative atom that has a lone pair of electrons), its directionality (i.e., it is stronger when the H atom is aligned with the two electronegative atoms), and its dynamic nature. This notwithstanding, the hydrogen bonds lose strength during hydration and may even be disrupted. We introduced a bulky adamantyl substituent in the UPy comonomer **4** with the aim of creating a hydrophobic structural milieu able to shield the hydrogen bonds from the water molecules.

Heterodimerization with 2,7-Bis(dodecanoylamino)−1,8-naphthyridine (5)

To characterize the copolymers of DMAA with **4** by routine techniques (SEC, NMR) or to determine the comonomers' reactivity ratios proved to be tasks of inordinate difficulty due to the poor solubility of the copolymers in common solvents and/or to the low amount of comonomer **4** that could be incorporated in the feed (because of its limited solubility). The solid state ^{13}C NMR spectroscopic analysis of the copolymers swollen in D_2O resulted in signals corresponding exclusively to DMAA. In the dried copolymers, only the signal corresponding to the endocyclic alkylidene carbon at 106 ppm was observed beside the DMAA signals.



Scheme 2. Disruption of homodimer **4-4** and formation of heterodimer **4–5**. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The 2,7-diamido-1,8-naphthyridines have been used successfully to investigate the competitive binding behavior of the molecules able to generate multiple-hydrogen dimmers.[55,57,110–118] Corbin and Zimmerman were the first to show[110] that these molecules were able to disrupt certain quadruply hydrogen-bonded homodimers and generate heterodimers through strong selective association processes and despite unfavorable secondary hydrogen-bond interactions. In the present study, we made use of this capability of the naphthyridines in order to estimate the proportion of monomer **4** which was covalently incorporated in the copolymer.

We investigated the naphthyridine **5** as a possible "solubilizing" agent that would enable the spectrometric quantification of the UPy moieties incorporated in copolymers. Compound **5** exhibits poor solubility in water but is soluble in chloroform. The addition in excess of **5** to homodimer **4-4** in $CDCl_3$ will result in formation of **4–5** heterodimers by the disruption of the homodimers via an ADDA-DAAD hydrogen bonding array, as shown in Scheme 2. The solubilization of gels is an indirect proof that in nonpolar organic solvents such as chloroform the existence of hydrogen bonds is responsible for the maintenance of a gel state. The process leads to the dissolution of gels, thus allowing their characterization by ^{1}H NMR spectroscopy.

A typical spectrum of the heterodimer system is shown in Figure 3. The signals at 8.2 and 8.5 ppm correspond to the aromatic protons "b" and "c" in **5**, which is in excess. Either "b" or "c" was employed as an internal standard and normalized to an integral value of 2, as each signal corresponds to two protons. The signal at 5.85 ppm corresponds to the alkylidene proton "a" at position C-5 in the pyrimidine heterocycle in compound **4**, and was integrated with respect to "b" (or "c") to result in values for I_U in eq. (1), which was used to calculate the mass fraction X_U of comonomer **4** in the copolymers.

$$X_U = I_U c_N V_N M_U / w_{\text{pol}} \quad (1)$$

Here, I_U is the integral of signal corresponding to protons "a" when the integral of proton "b" (or "c") is normalized to 2; c_N is the concentration of the solution of **5** in $CDCl_3$ (in this case 0.01M); V_N is the volume of solution of **5** required to solubilize a polymer sample of weight w_{pol}; and M_U is the molar mass of comonomer **4**.

The results are included in Table I. The concentration of comonomer **4** was lower in the experimentally determined composition than in the feed composition: ~1% versus 1.5% (**A**), ~2% versus 3% (**B**), ~3% versus 4.5% (**C**), and ~4% versus 5.9% (**D**).

Rheological Evaluation of the Self-Healing Capacity of Copolymers

Small-deformation rheometry is an essential tool in assessing and developing self-healing gels. Oscillatory shear experiments can reveal the dynamic mechanical properties of a gel (hydrogel, organogel) and may also serve as means not only to demonstrate the gel character, but also to confirm its self-healing characteristics (if the case). Essentially, the small deformations allow the properties of a sample to be measured without altering the structure of the material being tested. The result of such experiments, known as dynamic mechanical spectra, are plots of the real (G', the storage or elastic modulus) and imaginary (G'', the loss or viscous modulus) parts of the complex dynamic shear modulus (G^*) versus frequency (ν) or angular frequency (ω) of the oscillatory stress, usually displayed logarithmically. Based on Ross-Murphy's proposal,[119–121] which has become a rheological criterion for defining the state of a gel,[122] it is commonly accepted that in gels where the bonds have a permanent character, $G'(\omega)$ and $G''(\omega)$ show little dependence on frequency and always $G'(\omega) > G''(\omega)$, while in gels where the bonds have a temporary character (e. g. reversible bonds), there is a G'-G'' crossover marking the sol-gel transition, while $G'(\omega) < G''(\omega)$ (indicating free-flowing fluid) at low frequencies and $G'(\omega) > G''(\omega)$ (indicating resilient gel) at high frequencies.

The dynamic mechanical spectra in Figure 4 represent the evolution of G' and G'' as a function of the angular frequency of the oscillatory stress applied. Based on the above considerations, these spectra show that, within a frequency range between 0.1

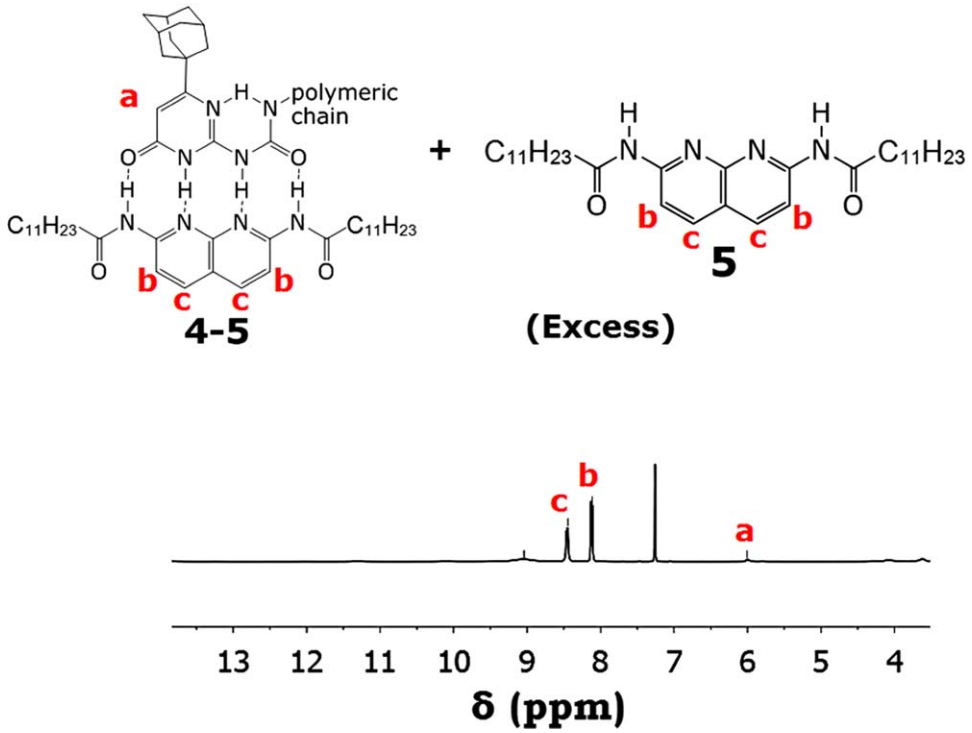


Figure 3. Selected region in the ^{1}H NMR spectrum of a copolymer (sample **B**) treated with excess of 2,7-*bis*(dodecanoylamino)−1,8-naphthyridine (**5**) in $CDCl_3$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and 100 rad/s, the copolymers are in a gel state and display solid-like behavior, both at the lowest (∼1%, copolymer **A**) and the highest (∼4%, copolymer **D**) contents in comonomer **4**, such indicating that the hydrogen bonding that is responsible for the formation of hydrogels has a permanent character. This assertion is valid at the concentrations of solid matter in hydrogels which were used in our assessment (i.e., 15% or higher).

To prove that the crosslinking through hydrogen bonds induces the formation of gels, we synthesized the *uncrosslinked* poly(*N,N′*-dimethylacrylamide) homopolymer and hydrated it at a concentration of 15% in water. The dynamic mechanical spectrum (Figure 5) indicates unequivocally a liquid-like behavior even in the absence of a shear-induced breakdown.

To assess the self-healing properties of the hydrated copolymers, the resulting hydrogels were extruded by injecting them through a 22-gauge (ID = 0.394 mm) syringe needle and then subjected to a fixed strain amplitude in the rheometer, at an angular frequency of 10 rad/s for 15 min. Samples **A** and **D** showed remarkable recovery following their extrusion through the needle (Figure 6). The slight increase of $G'(t)$ for the samples before and after being injected can be interpreted as a proof of recovery of the original mechanical properties of hydrogels after cessation of shear stress. This indicates that the shear stress generated upon injection did not affect significantly the network structure of hydrogels, likely as a result of the restoration of hydrogen bonds and the consequent re-assembling of the gel particles. Such behavior can be regarded as a valid experimental proof for the self-healing capacity of the copolymers. A higher content of comonomer **4** in copolymers leads to more extensive hydrogen bonding and consequently to stronger gel networks. Certain damage to the network is induced by the injection process, which is seen as a drop in the magnitude of G' and is especially significant in the copolymer with a higher content in

Table I. Formulation, Composition, and Molar Mass of the Copolymers of DMAA with Monomer **4**

	Monomer **4** in the feed		Content of **4** in copolymer[a]				
Sample	% wt	% mol	% wt	% mol	M_n[b] x 10^{-3} (g/mol)	M_w[b] x 10^{-3} (g/mol)	M_w/M_n
A	1.5	0.38	1.02	0.25	442.3	905.9	2.05
B	3.0	0.76	1.93	0.48	493.8	974.4	1.97
C	4.5	1.14	3.01	0.76	525.0	1125.0	2.14
D	5.9	1.52	4.02	1.03	424.0	992.1	2.34

[a] Estimated by the ^{1}H NMR analysis of the heterodimers formed with compound **5**.
[b] Determined by SEC.

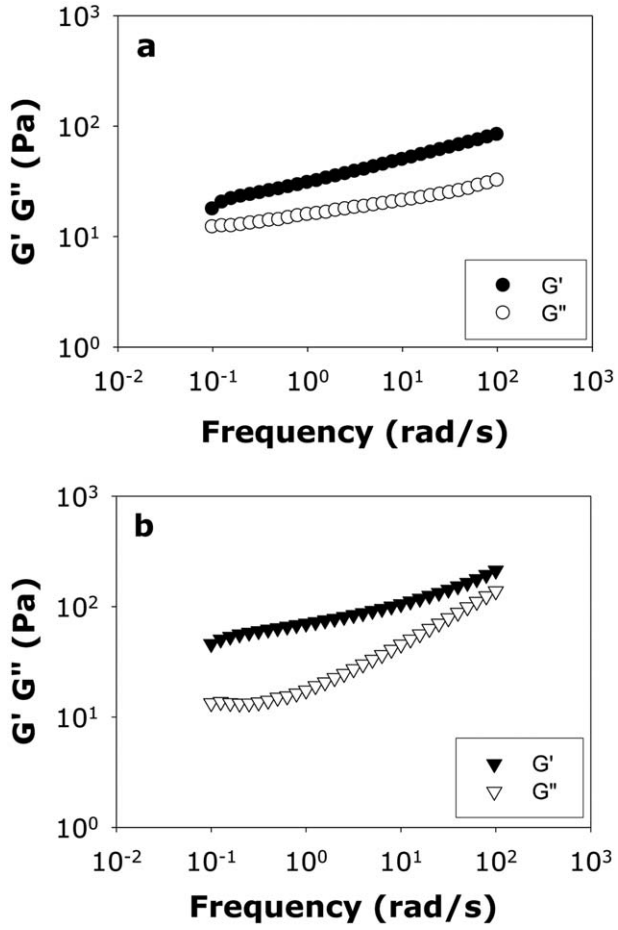


Figure 4. Dynamic mechanical spectra of the hydrated copolymers **A** (a) and **D** (b). The polymer content was 15%.

4 (sample **D**), but the material recovers well and maintains the character of a resilient gel. Figure 6 also shows that less hydrogen bonding in sample **A** is related to less network character, leading to a much lower magnitude of the elastic modulus as compared to that of sample **D**, and to almost identical values

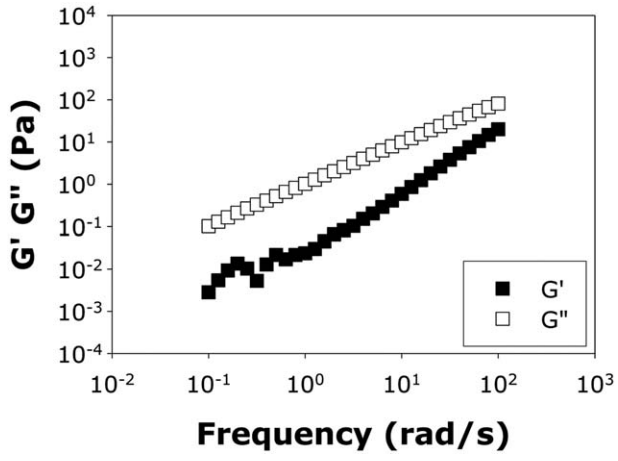


Figure 5. Dynamic mechanical spectrum of hydrated uncrosslinked poly(*N,N′*-dimethylacrylamide) homopolymer. The polymer content was 15%.

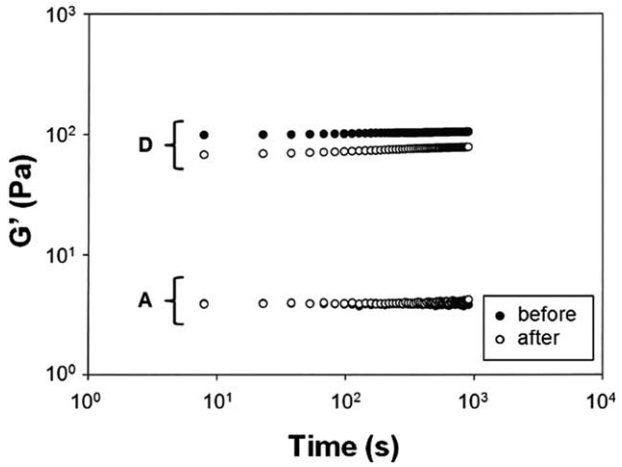


Figure 6. Variation with time of the storage modulus in the hydrogels **A** and **D** before and after extrusion through a 22-gauge syringe needle. The polymer content was 15%, and the frequency of the oscillatory shear stress was 10 rad/s.

before and after injection. Clearly, sample **A** tends to behave like a viscous fluid.

Brief Overview of Contact Self-Healing Hydrogels

It is the inherent nature of hydrogels that makes the study of self-healing hydrogels both specific and unique. We should recall that a gel can be defined as a state of matter consisting of a solid network which is able to entrap and retain large amounts of a liquid. The solid is generally insoluble in the penetrating liquid (although gels can be also generated by cooling diluted solutions). Thomas Graham introduced the term "hydrogel" for the silicic acid jelly formed in water,[123] and the term was extended to all gels that retain water.

There is no review published on self-healing *hydrogels* despite of a respectable number of published reports about the hydrogels able to display self-healing characteristics. As a notable exception, the self-assembling peptide-based hydrogels have been comparatively widely studied. Their formation is a result of a variety of physical interactions (hydrophobic, ionic, hydrogen bonds), and some of these hydrogels are able to re-assemble after shear-induced breakdown.[124–136]

It is worth to briefly summarize the main reported attempts to generate synthetic self-healing hydrogels. The first self-healing hydrogel ever reported was obtained by Schultz and Myers[137] by mixing aqueous solutions of sodium borate and poly(vinyl alcohol) (PVA). This was based on the well known complexation of monoborate ion, $B(OH)_4^-$, with polyhydroxylic compounds,[138–140] leading to the formation of reversible dipolar crosslinks that involve tetrahedral boron. Although the borate-hydrogels of PVA have been studied extensively,[141–152] an application based on their self-healing ability is yet to be reported.

Varghese et al. have shown[153] that certain hydrogels made by polymerization of *N*-acryloyl amino acids containing a hydrophobic alkylene chain (longer than C_5) between the N atom and the carboxyl group can display self-healing characteristics. When two pieces of hydrogel were immersed in a solution of

$CuCl_2$ and brought into mutual contact, they joined together in a process thought to be a metal–ion-mediated complexation through Cu^{2+}-carboxyl oxygen atoms dipolar (coordinative) crosslinks between two polymer chains. Similar systems based on ion-mediated dipolar crosslinks have been also reported.[154] Phadke et al. also reported[155] that the self-healing process could be achieved in covalently crosslinked hydrogels based on acryloyl-6-aminocaproic acid (C_6) without the intervention of metal ions, through hydrogen bonding between the amide and carboxyl groups. However, the process was effective only at $pH \leq 3$, which restricts significantly the applications of these hydrogels as biomaterials.

Self-healing properties have been demonstrated[156–158] in poly(-ethylene glycol) (PEG)-silicate "nanocomposites." The reversible crosslinking of PEG chains occurred via ionic interactions induced by the silicate lamellar nanoparticles (Laponite®, a synthetic silicate "clay" available commercially) through a mechanism not fully understood.[157] In systems developed subsequently along the same idea, poly(sodium acrylate), Laponite® and PEG-based dendrons possessing peripheral charged guanidinium groups were mixed in water and afforded re-mending hydrogels.[159] These developments were likely inspired by previous work on hydrogels based on poly(*N*-isopropylacrylamide) and a synthetic silicate clay (hectrite).[160]

An interesting self-healing system was reported[161] where the water was not detrimental to the hydrogen bonding process, actually being essential for hydrogelation, which has been explained through the chlorine-mediated hydrogen bonds between the polymer chains and water. Recently, self-healing hydrogel films have been created by the alternating layer-by-layer deposition of a covalently crosslinked anionic polymer (as a "microgel") and a cationic polymer containing quaternary amine moieties.[162] Hydrogels have been also reported where the self-healing characteristics appeared to be imparted by hydrophobic interactions.[163–166] Other re-mendable hydrogels can result from a process of molecular recognition in cyclodextrin systems.[167]

Open supramolecular assemblies based on UPy–end-functionalized PEGs have been recently developed as potential self-healing hydrogels.[168] Alkyl segments (C_6 to C_{12}) were inserted between UPy and additional ureido moieties to provide hydrophobic pockets and to promote lateral hydrogen bonding. At concentrations higher than 10% in water, the materials displayed gel-like rheology below 40°C. These transient hydrogels displayed nanofibrous structure and eroded in water relatively fast. Their erodibility, likely due to a progressive solubilization, was seen as an advantage when used as carriers for sustained drug delivery despite of a fast release rate. A hydrogel loaded with a bioactive protein (BMP7) was implanted in the rat kidney; after 7 days, there was no hydrogel left and the protein had been delivered in an active state. In another reported PEG-based open supramolecular assembly,[169] the hydrophobic shielding of the UPy-based hydrogen bonds was assured through isophorone groups. The resulting hydrogel was not injectable, but showed the capacity to re-mend upon pressure contact.

Recently, a self-healing-like approach has been suggested for vitreous substitution.[170] It is based on aqueous solutions of two polymers that, upon mixing, form a hydrogel through host-guest assembling between a polymeric β-cyclodextrin and a sulfonated acrylamide copolymer.

Significance of the Hydrogels Reported in this Study

It is obvious from the above brief overview, where we choose to be illustrative rather than comprehensive, that current research is mainly focused on the hydrogels that can self-heal by *physical contact*. Although involving a variety of non-covalent interactions, only two of the reported hydrogel systems[168,169] were based on self-assemblies induced by multiple-point hydrogen-bonding motifs, and only one was developed[168] as an implantable biomaterial. The other systems do not appear suitable as biomaterials; they are not injectable either.

The hydrogels described in the present study are transparent and can be injected through a syringe needle without losing their rheological characteristics or transparency, similar to those recently described elsewhere.[168] This behavior is a proof that the multiple hydrogen bonds in the self-assembled copolymers can be shielded effectively by the hydrophobic bulky adamantyl substituent, leading to the retention of the character of a gel (or of a viscoelastic fluid) if the amount of hydration water is not excessive. Such hydrogels can potentially function as vitreous substitutes in the vitreous cavity where the amount of water is limited and physiologically controlled. After administration through injection in the vitreous cavity, these hydrogels can recover and maintain their gel-like properties *precisely* because the available amount of water will not be sufficient to solubilize completely the material. Therefore, the hydrogels would be able to exert the necessary tamponade pressure in order to push the retina and maintain the subretinal space to its normal size. Also importantly, these hydrogels should not elicit toxic reaction to the eye tissues. Preliminary *in vitro* cytotoxic evaluation using sample **C** as a representative hydrogel has been carried out in our laboratory (unpublished results). Retinal pigment epithelial cells of the ARPE-19 line were grown in culture in the presence of pieces of the fully swollen hydrogel. Microscopically, the proliferating cells showed normal morphology after 4 days of contact with the hydrogel.

Whilst in the study discussed above,[168] the erodibility of the hydrogels was regarded as a useful characteristic due to the intended application, our hydrogels must not undergo erosion in water. They have to remain at the site of insertion (the eye's vitreous cavity in this case) for longer time and exert an internal pressure on the cavity's walls, which will happen only if they restore to a gel state or to highly viscous fluids.

We believe that the presence of the bulky adamantyl substituent at position 6 in the pyrimidine heterocycle contributed effectively to the protective shielding of the hydrogen-bonded crosslinks and survival of the gel-like structure in water, when the solid content in the hydrogels was 15% or higher. We assume that by introducing additional alkyl substituents in the UPy modules, further shielding and stabilization the hydrogen bonds can be provided. Such substituents may be introduced, for

instance, at position 5 in the pyrimidine heterocycle and/or at the carbon atom in the oxyethyl moiety attached directly (α-position) to the atom N' of the ureido group [(O=C) – NH – C(α) – CH_2 – O –].

CONCLUSIONS

The self-assembling of hydrophilic macromolecular chains by non-covalent crosslinking through hydrogen-bonded ureido-pyrimidinone motifs is a valid approach to create self-healing hydrogels, which are able to regenerate the hydrogen bonds by physical contact between the gel fragments after their mechanically induced breakdown. To be effective in aqueous media, this approach requires a bulky alkyl substituent in the UPy molecule in order to shield the hydrogen bonds against the surrounding water molecules. Even with a bulky substituent at position 6 in the pyrimidine heterocycle, there is a concentration threshold below which the copolymer dissolves. It can be assumed that certain structural modifications of the UPy monomers leading to enhanced shielding of the hydrogen bonds may lower this threshold.

ACKNOWLEDGMENTS

The study was supported by the Prevent Blindness Foundation through Viertel's Vision Program, Queensland, Australia, and by the Smart State Innovation Projects Fund, the Government of Queensland, Australia, through National and International Research Alliances Program. The authors thank Dr. Shuko Suzuki for helping with the editorial process.

REFERENCES

1. van der Zwaag, S. In Self Healing Materials; van der Zwaag, S., Ed.; Springer: Dordrecht, **2007**; p 1.
2. Trask, R. S.; Williams, H. R.; Bond, I. P. *Bioinsp. Biomim.* **2007**, *2*, P1.
3. Fratzl, P. J. R. *Soc. Interface* **2007**, *4*, 637.
4. Amendola, V.; Meneghetti, M. *Nanoscale* **2009**, *1*, 74.
5. Murphy, E. B.; Wudl, F. *Prog. Polym. Sci.* **2010**, *35*, 223.
6. Wool, R. P. *Soft Matter* **2008**, *4*, 400.
7. Andersson, H. M.; Keller, M. W.; Moore, J. S.; Sottos, N. R.; White, S. R. In Self Healing Materials; van der Zwaag, S., Ed.; Springer: Dordrecht, **2007**; p 19.
8. Bergman, S. D.; Wudl, F. In Self Healing Materials; van der Zwaag, S., Ed.; Springer: Dordrecht, **2007**; p 45.
9. Bergman, S. D.; Wudl, F. *J. Mater. Chem.* **2008**, *18*, 41.
10. Wu, D. Y.; Meure, S.; Solomon, D. *Prog. Polym. Sci.* **2008**, *33*, 479.
11. Blaiszik, B. J.; Kramer, S. L. B.; Olugebefola, S. C.; Moore, J. S.; Sottos, N. R.; White, S. R. *Annu. Rev. Mater. Res.* **2010**, *40*, 179.
12. Fredericks, J. R.; Hamilton, A. D. In Comprehensive Supramolecular Chemistry; Sauvage, J. P.; Hosseini, M. W., Eds.; Elsevier Science: Oxford, **1996**; Vol. 9, p 565.
13. Zimmerman, N.; Moore, J. S.; Zimmerman, S. C. *Chem. Ind.* **1998**, 604.
14. Moore, J. S. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 108.
15. Corbin, P. S.; Zimmerman, S. C. In Supramolecular Polymers; Ciferri, A., Ed.; Marcel Dekker: New York, **2000**; p 147.
16. Zimmerman, S. C.; Corbin, P. S. *Struct. Bonding* **2000**, *96*, 63.
17. Krische, M. J.; Lehn, J. -M. *Struct. Bonding* **2000**, *96*, 3.
18. Brunsveld, L.; Folmer, B. J. B.; Meijer, E. W.; Sijbesma, R. P. *Chem. Rev.* **2001**, *101*, 4071.
19. Sherrington, D. C.; Taskinen, K. A. *Chem. Soc. Rev.* **2001**, *30*, 83.
20. Prins, L. J.; Reinhoudt, D. N.; Timmerman, P. *Angew. Chem. Int. Ed.* **2001**, *40*, 2382.
21. Hofmeier, H.; Schubert, U. S. *Chem. Commun.* **2005**, 2423.
22. Armstrong, G.; Buggy, M. *J. Mater. Sci.* **2005**, *40*, 547.
23. Binder, W. H.; Zirbs, R. *Adv. Polym. Sci.* **2007**, *207*, 1.
24. ten Brinke, G.; Ruokolainen, J.; Ikkala, O. *Adv. Polym. Sci.* **2007**, *207*, 113.
25. Wilson, A. J. *Soft Matter* **2007**, *3*, 409.
26. Dankers, P. Y. W.; Meijer, E. W. *Bull. Chem. Soc. Jpn* **2007**, *80*, 2047.
27. de Greef, T. F. A.; Meijer, E. W. *Nature* **2008**, *453*, 171.
28. de Greef, T. F. A.; Smulders, M. M. J.; Wolffs, M.; Schenning, A. P. H. J.; Sijbesma, R. P.; Meijer, E. W. *Chem. Rev.* **2009**, *109*, 5687.
29. Lange, R. F. M.; van Gurp, M.; Meijer, E. W. *J. Polym. Sci. Part A: Polym. Chem.* **1999**, *37*, 3657.
30. Hirschberg, J. H. K. K.; Brunsveld, L.; Ramzi, A.; Vekemans, J. A. J. M.; Sijbesma, R. P.; Meijer, E. W. *Nature* **2000**, *407*, 167.
31. Wang, G.; Hamilton, A. D. *Chem. Commun.* **2003**, 310.
32. Makarević, J.; Jokić, M.; Perić, B.; Tomišić, V.; Kojić-Prodić, B.; Žinić, M. *Chem. Eur. J.* **2001**, *7*, 3328.
33. Xing, B.; Yu, C. -W.; Chow, K. -H.; Ho, P. -L.; Fu, D.; Xu, B. *J. Am. Chem. Soc.* **2002**, *124*, 14846.
34. Zhang, Y.; Gu, H.; Yang, Z.; Xu, B. *J. Am. Chem. Soc.* **2003**, *125*, 13680.
35. Suzuki, M.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *New J. Chem.* **2002**, *26*, 817.
36. Suzuki, M.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Chem. Eur. J.* **2003**, *9*, 348.
37. Suzuki, M.; Yumoto, M.; Kimura, M.; Shirai, H.; Hanabusa, K. *Helv. Chim. Acta* **2003**, *86*, 2228.
38. van Bommel, K. J. C.; van der Pol, C.; Muizebelt, I.; Friggeri, A.; Heeres, A.; Meetsma, A.; Feringa, B. L.; van Esch, J. *Angew. Chem. Int. Ed.* **2004**, *43*, 1663.
39. Mahajan, S. S.; Paranji, R.; Mehta, R.; Lyon, R. P.; Atkins, W. M. *Bioconjugate Chem.* **2005**, *16*, 1019.
40. Zhou, Y.; Yi, T.; Li, T.; Zhou, Z.; Li, F.; Huang, W.; Huang, C. *Chem. Mater.* **2006**, *18*, 2974.
41. Das, D.; Dasgupta, A.; Roy, S.; Mitra, R. N.; Debnath, S.; Das, P. K. *Chem. Eur. J.* **2006**, *12*, 5068.
42. Xie, Z.; Zhang, A.; Ye, L.; Feng, Z. *Soft Matter* **2009**, *5*, 1474.

43. Obert, E.; Bellot, M.; Bouteiller, L.; Andrioletti, F.; Lehen-Ferrenbach, C.; Boué, F. *J. Am. Chem. Soc.* **2007**, *129*, 15601.
44. Chirila, T. V.; Hong, Y.; Dalton, P. D.; Constable, I. J.; Refojo, M. F. *Prog. Polym. Sci.* **1998**, *23*, 475.
45. Swindle-Reilly, K. E.; Ravi, N. In Biomaterials and Regenerative Medicine in Ophthalmology; Chirila, T., Ed.; Woodhead Publishing: Oxford, **2010**; p 339.
46. Kleinberg, T. T.; Tzekov, R. T.; Stein, L.; Ravi, N.; Kaushal, S. *Surv. Ophthalmol.* **2011**, *56*, 300.
47. Hong, Y.; Chirila, T. V.; Vijayasekaran, S.; Dalton, P. D.; Tahija, S. G.; Cuypers, M. J. H.; Constable, I. J. *J. Biomed. Mater. Res.* **1996**, *30*, 441.
48. Vijayasekaran, S.; Chirila, T. V.; Hong, Y.; Tahija, S. G.; Dalton, P. D.; Constable, I. J.; McAllister, I. L. *J. Biomater. Sci. Polym. Ed.* **1996**, *7*, 685.
49. Sijbesma, R. P.; Beijer, F. H.; Brunsveld, L.; Folmer, B. J. B.; Hirschberg, J. H. K. K.; Lange, R. F. M.; Lowe, J. K. L.; Meijer, E. W. *Science* **1997**, *278*, 1601.
50. Beijer, F. H.; Sijbesma, R. P.; Kooijman, H.; Spek, A. L.; Meijer, E. W. *J. Am. Chem. Soc.* **1998**, *120*, 6761.
51. Söntjens, S. H. M.; Sijbesma, R. P.; van Genderen, M. H. P.; Meijer, E. W. *J. Am. Chem. Soc.* **2000**, *122*, 7487.
52. Folmer, B. J. B.; Sijbesma, R. P.; Versteegen, R. M.; van der Rijt, J. A. J.; Meijer, E. W. *Adv. Mater.* **2000**, *12*, 874.
53. Sijbesma, R. P.; Meijer, E. W. *Chem. Commun.* **2003**, *5*.
54. Keizer, H. M.; van Kessel, R.; Sijbesma, R. P.; Meijer, E. W. *Polymer* **2003**, *44*, 5505.
55. de Greef, T. F. A.; Nieuwenhuizen, M. M. L.; Sijbesma, R. P.; Meijer, E. W. *J. Org. Chem.* **2010**, *75*, 598.
56. Nieuwenhuizen, M. M. L.; de Greef, T. F. A.; van der Bruggen, R. L. J.; Pauluse, J. M. J.; Appel, W. P. J.; Smulders, M. M. J.; Sijbesma, R. P.; Meijer, E. W. *Chem. Eur. J.* **2010**, *16*, 1601.
57. Ligthart, G. B. W. L.; Ohkawa, H.; Sijbesma, R. P.; Meijer, E. W. *J. Am. Chem. Soc.* **2005**, *127*, 810.
58. Armarego, W. L. F.; Chai, C. L. L. Purification of Laboratory Chemicals, 5th ed.; Elsevier Science/Butterworth Heinemann: Amsterdam, **2003**; p 435.
59. van Beek, D. J. M.; Spiering, A. J. H.; Peters, G. W. M.; te Nijenhuis, K.; Sijbesma, R. P. *Macromolecules* **2007**, *40*, 8464.
60. Gruijters, B. W. T.; Broeren, M. A. C.; van Delft, F. L.; Sijbesma, R. P.; Hermkens, P. H. H.; Rutjes, F. P. J. T. *Org. Lett.* **2006**, *8*, 3163.
61. Overberger, C. G.; Kogon, I. C. *J. Am. Chem. Soc.* **1954**, *76*, 1879.
62. Giner-Sorolla, A.; Bendich, A. *J. Am. Chem. Soc.* **1958**, *80*, 5744.
63. Baker, B. R.; Santi, D. V.; Almaula, P. I.; Werkheiser, W. C. *J. Med. Chem.* **1964**, *7*, 24.
64. Hong, C. I.; Piantadosi, C.; Chae, C. B.; Irvin, J. L. *J. Med. Chem.* **1968**, *11*, 1182.
65. Jonak, J. P.; Zakrzewski, S. F.; Mead, L. H.; Hakala, M. T. *J. Med. Chem.* **1970**, *13*, 1170.
66. Jonak, J. P.; Zakrzewski, S. F.; Mead, L. H. *J. Med. Chem.* **1972**, *15*, 662.
67. Behrend, R. *Ber. Deut. Chem. Ges.* **1886**, *19*, 219.
68. Behrend, R. *Liebigs Ann. Chem.* **1886**, *233*, 2.
69. Curatolo, T. *Gazz. Chim. Ital.* **1890**, *20*, 585.
70. Jaeger, J. *Liebigs Ann. Chem.* **1891**, *262*, 365.
71. Falco, E. A.; Russell, P. B.; Hitchings, G. H. *J. Am. Chem. Soc.* **1951**, *73*, 3753.
72. Hyde, K. A.; Acton, M.; Baker, B. R.; Goodman, L. *J. Org. Chem.* **1962**, *27*, 1717.
73. Baker, B. R.; Sachdev, K. *J. Pharm. Sci.* **1964**, *53*, 1020.
74. Baker, B. R.; Shapiro, H. S. *J. Pharm. Sci.* **1966**, *55*, 308.
75. Hlavka, J. J.; Bitha, P.; Lin, Y.; Strohmeyer, T. *J. Heterocyclic Chem.* **1984**, *21*, 1537.
76. Yamauchi, K.; Lizotte, J. R.; Long, T. E. *Macromolecules* **2003**, *36*, 1083.
77. Elkins, C. L.; Park, T.; McKee, M. G.; Long, T. E. *J. Polym. Sci. Part A: Polym. Chem.* **2005**, *43*, 4618.
78. Li, J.; Viveros, J. A.; Wrue, M. H.; Anthamatten, M. *Adv. Mater.* **2007**, *19*, 2851.
79. Pfeiffer, P.; von Modelski, J. *Hoppe-Seyler's Z. Physiol. Chem.* **1912**, *81*, 329.
80. Pfeiffer, P.; von Modelski, J. *Hoppe-Seyler's Z. Physiol. Chem.* **1913**, *85*, 1.
81. Pfeiffer, P. *Hoppe-Seyler's Z. Physiol. Chem.* **1924**, *133*, 22.
82. Ambrose E. J.; Bamford, C. H.; Elliot, A.; Hanby, W. E. *Nature* **1951**, *167*, 264.
83. Bair, T. I.; Morgan, P. W.; Killian, F. L. *Macromolecules* **1977**, *10*, 1396.
84. McCormick, C. L.; Lichatowich, D. K. *J. Polym. Sci. Polym. Lett. Ed.* **1979**, *17*, 479.
85. Seebach, D.; Thaler, A.; Beck, A. K. *Helv. Chim. Acta* **1989**, *72*, 857.
86. Thaler, A.; Seebach, D.; Cardinaux, F. *Helv. Chim. Acta* **1991**, *74*, 628.
87. Köck, M.; Kessler, H.; Seebach, D.; Thaler, A. *J. Am. Chem. Soc.* **1992**, *114*, 2676.
88. Bromberg, L. *J. Phys. Chem.* **1994**, *98*, 10628.
89. Gagnaire, D.; Saint-Germain, J.; Vincendon, M. *J. Appl. Polym. Sci. Appl. Polym. Symp.* **1983**, *37*, 261.
90. Rao, C. N. R.; Bhujle, V. V.; Goel, A.; Bhat, U. R.; Paul, A. *J. Chem. Soc. Chem. Commun.* **1973**, 161.
91. Rao, C. N. R.; Rao, K. G.; Balasubramanian, D. *FEBS Lett.* **1974**, *46*, 192.
92. Rao, K. G.; Becker, E. D.; Rao, C. N. R. *J. Chem. Soc. Chem. Commun.* **1977**, 350.
93. Saint-Germain, J.; Vincendon, M. *Org. Magn. Reson.* **1983**, *21*, 371.
94. Seebach, D. *Aldrichim. Acta* **1992**, *25*, 59.
95. Ciferri, A. In Supramolecular Polymers; Ciferri, A., Ed.; Marcel Dekker: New York, **2000**; p 1.
96. Ciferri, A. *Macromol. Rapid Commun.* **2002**, *23*, 511.

97. Ciferri, A. In Supramolecular Polymers, 2nd ed.; Ciferri, A., Ed.; CRC Press/ Taylor & Francis Group: Boca Raton, **2005**; p 29.
98. Yount, W. C.; Loveless, D. M.; Craig, S. L. *J. Am. Chem. Soc.* **2005**, *127*, 14488.
99. Xu, J.; LaBean, T. H.; Craig, S. L. In Supramolecular Polymers, 2nd ed.; Ciferri, A., Ed.; CRC Press/Taylor & Francis Group: Boca Raton, **2005**; p 445.
100. Kato, T.; Kihara, H.; Kumar, U.; Uryu, T.; Fréchet, M. J. *Angew. Chem. Int. Ed.* **1994**, *33*, 1644.
101. Kato, T.; Fréchet, M. J. *Macromol. Symp.* **1995**, *98*, 311.
102. Kato, T. In Supramolecular Polymers; 2nd ed.; Ciferri, A., Ed.; CRC Press/Taylor & Francis Group: Boca Raton, **2005**; p 131.
103. Hirst, A. R.; Smith, D. K. *Langmuir* **2004**, *20*, 10851.
104. Yount, W. C.; Loveless, D. M.; Craig, S. L. *Angew. Chem. Int. Ed.* **2005**, *44*, 2746.
105. McKee, M. G.; Elkins, C. L.; Long, T. E. *Polymer* **2004**, *45*, 8705.
106. McKee, M. G.; Elkins, C. L.; Park, T.; Long, T. E. *Macromolecules* **2005**, *38*, 6015.
107. Yamauchi, K.; Lizotte, J. R.; Hercules, D. M.; Vergne, M. J.; Long, T. E. *J. Am. Chem. Soc.* **2002**, *124*, 8599.
108. Elkins, C. L.; Viswanathan, K.; Long, T. E. *Macromolecules* **2006**, *39*, 3132.
109. Mather, B. D.; Elkins, C. L.; Beyer, F. L.; Long, T. E. *Macromol. Rapid Commun.* **2007**, *28*, 1601.
110. Corbin, P. S.; Zimmerman, S. C. *J. Am. Chem. Soc.* **1998**, *120*, 9710.
111. Park, T.; Todd, E. M.; Nakashima, S.; Zimmerman, S. C. *J. Am. Chem. Soc.* **2005**, *127*, 18133.
112. Zhao, X.; Wang, X. -Z.; Jiang, X. -K.; Chen, Y. -Q.; Li, Z. -T.; Chen, G. -J. *J. Am. Chem. Soc.* **2003**, *125*, 15128.
113. Wang, X. -Z.; Li, X. -Q.; Shao, X. -B.; Zhao, X.; Deng, P.; Jiang, X. -K.; Li, Z. -T.; Chen, Y. -Q. *Chem. Eur. J.* **2003**, *9*, 2904.
114. Li, X. -Q.; Jiang, X. -K.; Wang, X. -Z.; Li, Z. -T. *Tetrahedron* **2004**, *60*, 2063.
115. Li, X. -Q.; Feng, D. -J.; Jiang, X. -K.; Li, Z. -T. *Tetrahedron* **2004**, *60*, 8275.
116. Scherman, O. A.; Ligthart, G. B. W. L.; Sijbesma, R. P.; Meijer, E. W. *Angew. Chem. Int. Ed.* **2006**, *45*, 2072.
117. de Greef, T. F. A.; Ligthart, G. B. W. L.; Lutz, M.; Spek, A. L.; Meijer, E. W.; Sijbesma, R. P. *J. Am. Chem. Soc.* **2008**, *130*, 5479.
118. de Greef, T. F. A.; Ercolani, G.; Ligthart, G. B. W. L.; Meijer, E. W.; Sijbesma, R. P. *J. Am. Chem. Soc.* **2008**, *130*, 13755.
119. Ross-Murphy, S. B. *Food Hydrocoll.* **1987**, *1*, 485.
120. Ross-Murphy, S. B. *Polym. Gels Netw.* **1994**, *2*, 229.
121. Kavanagh, G. M.; Ross-Murphy, S. B. *Prog. Polym Sci.* **1998**, *23*, 533.
122. Almdal, K.; Dyre, J.; Hvidt, S.; Kramer, O. *Polym. Gels Netw.* **1993**, *1*, 5.
123. Graham, T. *J. Chem. Soc.* **1864**, *17*, 318.
124. Schneider, J. P.; Pochan, D. J.; Ozbas, B.; Rajagopal, K.; Pakstis, L.; Kretsinger, J. *J. Am. Chem. Soc.* **2002**, *124*, 15030.
125. Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature* **2002**, *417*, 424.
126. Haines-Butterick, L.; Rajagopal, K.; Branco, M.; Salick, D.; Rughani, R.; Pilarz, M.; Lamm, S.; Pochan, D. J.; Schneider, J. P. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 7791.
127. Branco, M. C.; Schneider, J. P. *Acta Biomater.* **2009**, *5*, 817.
128. Ramachandran, S.; Tseng, Y.; Yu, Y.B. *Biomacromolecules* **2005**, *6*, 1316.
129. Deming, T. J. *Soft Matter* **2005**, *1*, 28.
130. Wong Po Foo, C. T. S.; Lee, J. S.; Mulyasasmita, W.; Parisi-Amon, A.; Heilshorn, S. C. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 22067.
131. Hirst, A. R.; Escuder, B.; Miravet, J. F.; Smith, D. K. *Angew. Chem. Int. Ed.* **2008**, *47*, 8002.
132. Kopeček, J.; Yang, J. *Acta Biomater.* **2009**, *5*, 805.
133. Bowerman, C. J.; Nilsson, B. L. *J. Am. Chem. Soc.* **2010**, *132*, 9526.
134. Zhao, X.; Wang, S.; Lu, Y.; Cheng, J. In Biologically-Responsive Hybrid Materials; Jabbari, E.; Khademhosseini, A., Eds.; World Scientific: Singapore, **2010**; p 83.
135. Ye, Z.; Elisseeff, J. In Biologically-Responsive Hybrid Materials; Jabbari, E.; Khademhosseini, A., Eds.; World Scientific: Singapore, **2010**; p 311.
136. Skrzeszewska, P. J.; Sprakel, J.; de Wolf, F. A.; Fokkink, R.; Cohen Stuart, M. A.; van der Gucht, J. *Macromolecules* **2010**, *43*, 3542.
137. Schultz, R. K.; Myers, R. R. *Macromolecules* **1969**, *2*, 281.
138. Böeseken, J. *Adv. Carbohydr. Chem.* **1949**, *4*, 189.
139. Deuel, H.; Neukom, H. *Makromol. Chem.* **1949**, *3*, 13.
140. Roy, G. L.; Laferriere, A. L.; Edwards, J. O. *J. Inorg. Nucl. Chem.* **1957**, *4*, 106.
141. Sinton, S. W. *Macromolecules* **1987**, *20*, 2430.
142. Shibayama, M.; Sato, M.; Kimura, Y.; Fujiwara, H.; Nomura, S. *Polymer* **1988**, *29*, 336.
143. Shibayama, M.; Yoshizawa, H.; Kurokawa, H.; Fujiwara, H.; Nomura, S. *Polymer* **1988**, *29*, 2066.
144. Kurokawa, H.; Shibayama, M.; Ishimaru, T.; Nomura, S. *Polymer* **1992**, *33*, 2182.
145. Shibayama, M.; Kurokawa, H.; Nomura, S.; Muthukumar, M.; Stein, R. S.; Roy, S. *Polymer* **1992**, *33*, 2883.
146. Leibler, L.; Pezron, E.; Pincus, P. A. *Polymer* **1988**, *29*, 1105.
147. Pezron, E.; Leibler, L.; Ricard, A.; Lafuma, F.; Audebert, R. *Macromolecules* **1989**, *22*, 1169.
148. Keita, G.; Ricard, A. *Polym. Bull.* **1990**, *24*, 633.
149. Keita, G.; Ricard, A.; Audebert, R.; Pezron, E.; Leibler, L. *Polymer* **1995**, *36*, 49.
150. Koike, A.; Nemoto, N.; Inoue, T.; Osaki, K. *Macromolecules* **1995**, *28*, 2339.

151. Chen, C. Y.; Yu, T. -L. *Polymer* **1997**, *38*, 2019.
152. Robb, I. D.; Smeulders, J. B. A. F. *Polymer* **1997**, *38*, 2165.
153. Varghese, S.; Lele, A.; Mashelkar, R. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, *44*, 666.
154. Kersey, K. R.; Loveless, D. M.; Craig, S. L. *J. R. Soc. Interface* **2007**, *4*, 373.
155. Phadke, A.; Zhang, C.; Arman, B.; Hsu, C. -C.; Mashelkar, R. A.; Lele, A. K.; Tauber, M. J.; Arya, G.; Varghese, S. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 4383.
156. Loizou, E.; Butler, P.; Porcar, L.; Schmidt, G. *Macromolecules* **2006**, *39*, 1614.
157. Schexnailder, P.; Schmidt, G. *Colloid Polym. Sci.* **2009**, *287*, 1.
158. Jin, Q.; Schexnailder, P.; Gaharwar, A. K.; Schmidt, G. *Macromol. Biosci.* **2009**, *9*, 1028.
159. Wang, Q.; Mynar, J. L.; Yoshida, M.; Lee, E.; Lee, M.; Okuro, K.; Kinbara, K.; Aida, T. *Nature* **2010**, *463*, 339.
160. Haraguchi, K.; Takehisa, T. *Adv. Mater.* **2002**, *14*, 1120.
161. Kundu, S. K.; Matsunaga, T.; Yoshida, M.; Shibayama, M. *J. Phys. Chem. B* **2008**, *112*, 11537.
162. South, A. B.; Lyon, L. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 767.
163. Jiang, G.; Liu, C.; Liu, X.; Zhang, G.; Yang, M.; Liu, F. *Macromol. Mater. Eng.* **2009**, *294*, 815.
164. Jiang, G.; Liu, C.; Liu, X.; Zhang, G.; Yang, M.; Chen, Q.; Liu, F. *J. Macromol. Sci. Part A: Pure Appl. Chem.* **2010**, *47*, 335.
165. Jiang, G.; Liu, C.; Liu, X.; Chen, Q.; Zhang, G.; Yang, M.; Chen, Q.; Liu, F. *Polymer* **2010**, *51*, 1507.
166. Tuncaboylu, D. C.; Sari, M.; Oppermann, W.; Okay, O. *Macromolecules* **2011**, *44*, 4997.
167. Harada, A.; Kobayashi, R.; Takashima, Y.; Hashidzume, A.; Yamaguchi, H. *Nature Chem.* **2011**, *3*, 34.
168. Dankers, P. Y. W.; Hermans, T. M.; Baughman, T. W.; Kamikawa, Y.; Kieltyka, R. E.; Bastings, M. M. C.; Janssen, H. M.; Sommerdijk, N. A. J. M.; Larsen, A.; van Luyn, M. J. A.; Bosman, A. W.; Popa, E. R.; Fytas, G.; Meijer, E. W. *Adv. Mater.* **2012**, *24*, 2703.
169. van Gemert, G. M. L.; Peeters, J. W.; Söntjens, S. H. M.; Janssen, H. M.; Bosman, A. W. *Macromol. Chem. Phys.* **2012**, *213*, 234.
170. Böhm, I.; Strotmann, F.; Koopmans, C.; Wolf, I.; Galla, H. -J.; Ritter, H. *Macromol. Biosci.* **2012**, *12*, 432.