Well-defined synthetic polymers with a protein-like gelation behavior in water†

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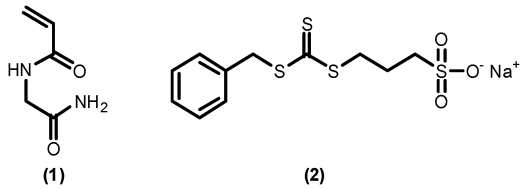
Homopolymers of *N*-acryloyl glycinamide were prepared by reversible addition-fragmentation chain transfer polymerization in water. The formed macromolecules exhibit strong polymer–polymer interactions in aqueous milieu and therefore form thermoreversible physical hydrogels in pure water, physiological buffer or cell medium.

Thermoreversible hydrogels are hydrophilic macromolecular networks exhibiting a thermo-induced sol–gel (*i.e.* the gel forms upon heating) or gel–sol (*i.e.* the gel melts upon heating) transition. Both types of gels have found wide applications in materials science, food science, cosmetics and medicine.[1,2] However, so far, the large majority of synthetic thermogels exhibit a sol–gel transition in water.[2] This interesting phenomenon can be obtained, for example, by using amphiphilic block copolymers[3] or macromolecular architectures containing thermoresponsive segments (*i.e.* polymers exhibiting a lower critical solution temperature in water).[4]

In contrast, biopolymer gels (*e.g.* protein-based or polysaccharide-based hydrogels) often exhibit a gel–sol behavior in aqueous medium.[5] Although quite common in our daily lives, this gelation mode is far from being trivial in terms of macromolecular science. It implies that (*i*) strong polymer–polymer interactions are formed at room temperature in water, (*ii*) the macromolecular network remains overall highly hydrophilic, and (*iii*) the physical crosslinks are weak enough to be disrupted by heating. In biopolymer gels, this subtle balance of parameters is due to the formation of highly-ordered crystallites. For instance, the gel–sol transition in protein gels frequently relies on the thermal disruption of helical aggregates.[6] This complex aggregation/disaggregation mechanism is based on specific peptide domains with a defined primary and secondary structure.

Comparable gel–sol transitions are rarely observed in synthetic hydrogels, which typically exhibit a less controlled macromolecular structure. Although this behavior has been observed in water for some polymer complexes or mixtures,[7] such multicomponent systems are usually difficult to tune for practical applications in materials science or life science.

In the present communication, we report that a simple homopolymer, prepared *via* controlled radical polymerization, exhibits an adjustable gel–sol transition in water and in physiological media. Polymers of *N*-acryloyl glycinamide (NAGAM, **1**, Scheme 1) are known to form strong self-interactions in aqueous medium, even at very low concentrations.[8] This is a very special situation implying that, while hydrophilic, this polymer has a higher tendency to form interactions with itself than with water.[9] This feature is exceptional among the class of uncharged water-soluble polymers. In most other cases (*e.g.* poly(ethylene oxide), poly(vinyl alcohol), poly(*N*-isopropylacrylamide)), polymer–water hydrogen bonding predominates.



(1) (2)

Scheme 1 Molecular structures of the monomer and the chain-transfer agent used in the present work.

The controlled radical polymerization of **1** was studied. After some preliminary investigations, it became obvious that the synthesis and characterization of poly(*N*-acryloyl glycinamide) are far from trivial. Indeed, the unconventional molecular structure of this polymer significantly limits the use of common organic solvents. Typically, homogeneous reaction conditions can only be obtained in water or in DMSO. Thus, the polymerization of **1** was investigated in pure deionized water. The reversible addition-fragmentation chain transfer (RAFT) process was selected among the different methods of controlled radical polymerization, as it is certainly the most adapted approach for aqueous polymerization.[10] The polymerizations were performed at 60 °C in the presence of the RAFT chain transfer agent **2** and a water-soluble azo radical initiator. A series of polymers with an average degree of polymerization ranging from 100 to 500 was synthesized (Table S1†). After synthesis and purification, the polymers formed were characterized by size exclusion chromatography (SEC) and NMR spectroscopy (Fig. 1).

^{1}H NMR spectra in D_2O clearly indicated that the polymers are end-functional structures obtained *via* a controlled RAFT process. Typical α- and ω-chain-end signals due to the initiating and control moieties of the chain transfer agent are seen in all spectra. The presence of defined chain-ends was also confirmed by Heteronuclear Single Quantum Coherence (Fig. S1†) and Heteronuclear Multiple Bond Coherence (data not shown) experiments. Moreover, a comparison of the integrals of the chain-end and backbone protons indicated a precisely controlled chain-length in all cases (Table S1†). This aspect was confirmed by the SEC measurements in hot DMSO.

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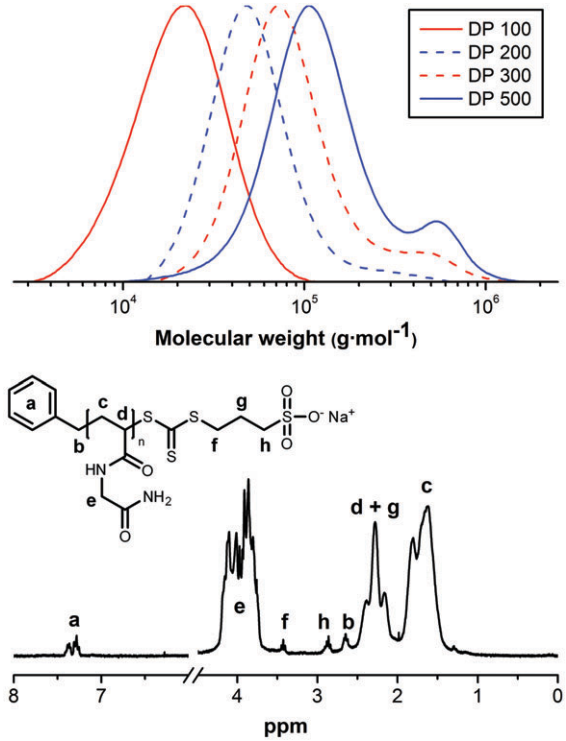

Fig. 1 Molecular characterization of homopolymers of **1** prepared by RAFT polymerization: (top) SEC chromatograms recorded at 70 °C in DMSO for samples of different DP_n; (bottom) ^{1}H NMR spectrum recorded in D_2O for a homopolymer with an average chain length of 100.

Chromatograms with a narrow molecular weight distribution were measured for each targeted degree of polymerization. Yet, it should be noted that a careful sample preparation is needed to molecularly dissolve the polymers (Fig. S2†). Due to the strong aggregation tendency of these macromolecules (Fig. S3†), high molecular weight shoulders may be observed in the SEC chromatograms. For high molecular weight samples (*i.e.* $DP_n > 300$), these aggregate peaks could be minimized but not completely suppressed (Fig. S2†). Nevertheless, both NMR and SEC indicated an efficient control of the macromolecular structure.

The properties of the obtained polymers have been investigated in aqueous milieu. All polymers formed physical gels at room temperature in pure water but also in phosphate buffered saline (PBS) solution and in cell medium (the last two media were selected in order to show the broad applicability of these materials). However, the critical gelation concentration (CGC) depended on polymer chain length (Fig. 2, inset). For instance, short polymer chains (*i.e.* $DP_n \sim 100$–200) did not form gels in PBS at concentrations lower than 10 wt%. On the other hand, samples with longer chains (*i.e.* $DP_n \sim 300$–500) formed gels at relatively low concentrations. For example, a homopolymer with an average chain length of 500 formed gels in PBS at concentrations as low as 5 wt%. Furthermore, a protein-like thermoreversible gelation behavior was observed in all cases. For example, Fig. 2 shows the rheological measurements of a PBS solution of a homopolymer with a DP_n of 500. A clear gel–sol transition is observed at 27 °C. This material forms a

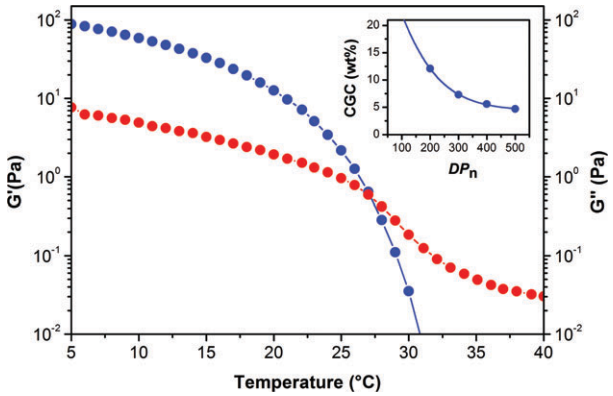

Fig. 2 Evolution of the storage modulus (G′, blue symbols) and the loss modulus (G′′, red symbols) as a function of temperature for a homopolymer of **1** ($DP_n = 500$) in phosphate buffered saline solution (concentration ~ 5.5 wt%). These measurements have been recorded at a frequency of 1 Hz. The inset shows the evolution of the critical gelation concentration as a function of DP_n for the polymers listed in Table S1†.

strong gel at room temperature but fully melts at physiological temperature.[11]

In conclusion, well-defined thermoreversible hydrogelators can be obtained *via* simple RAFT homopolymerization in aqueous medium. Similarly to proteins such as gelatin, these polymers form strong self-associations in water, which can be disrupted at elevated temperatures. These macromolecules are not only relevant for hydrogel applications, but more generally for the whole field of aqueous polymer self-assembly. Indeed, using controlled radical polymerization techniques,[10,12] a wide variety of self-associating polymer architectures may be envisioned and prepared.

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