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Molecular Nanofibers of Olsalazine Confer Supramolecular Hydrogels for Reductive Release of An Anti-inflammatory Agent

Xinming Li, **Jiayang Li**, **Yuan Gao**, **Yi Kuang**, **Junfeng Shi**, and **Bing Xu**[*]
Department of Chemistry, Brandeis University, 415 South St., Waltham, MA 02454, USA

Abstract

Tripeptide derivatives to conjugate with olsalazine, a clinically used anti-inflammatory prodrug, yield small molecules that self-assemble in water, which confer supramolecular hydrogels that undergo sol-gel phase transition upon reduction, resulting in the controlled-release of 5-aminosalicylic acid as the anti-inflammatory agent. This methodology will ultimately lead to new biomaterials for site-specific drug delivery.

The paper describes a supramolecular hydrogel as a potential biomaterial for site-specific drug release. Biomaterials derived from synthetic or biological polymeric hydrogels have found widespread applications in biomedical engineering, ranging from tissue repair, regenerative medicine, to drug delivery.[1] These polymer-based hydrogels, however, still have several inherent shortcomings, such as relatively slow degradation, unintended immune responses, and the generation of undesirable by-products.[2] On the other hand, supramolecular hydrogels,[3] formed by low molecular weight gelators[4] that self-assemble in water through non-covalent interactions, have attracted considerable attention because they exhibit several unique merits, such as synthetic economy, biocompatibility, low toxicity, inherent biodegradability, and, more importantly, fast thermally reversible formation-dissociation processes.[5] These advantages make supramolecular hydrogels a promising alternative for polymeric hydrogels. Among the molecules act as the building blocks for supramolecular hydrogels, peptide-based hydrogelators[6] are usual candidates because of their biological relevance, well-established synthetic chemistry (i.e., solid phase synthesis),[7] and the capability to produce a large set of diverse molecules from a small array of residues. There are many examples of peptide-based functional building blocks for making nanofibers and generating hydrogels. The nanofibers of peptide amphiphile (PA) molecules can display a high density of epitopes for regulating the differentiation of neuron progenitor cells[8] or guiding cartilage regeneration.[9] A supramolecular hydrogel self-assembled from lysine-containing short peptides exhibits inherent antibacterial activity.[10] Self-complementary oligopeptides form the hydrogels for cell culture and cytokine release.[11] Amino acid functionalized hydrogel particles release protein when triggered enzymatically.[12] A small peptide to conjugate with β-lactam transforms into a hydrogelator by the catalysis of a β-lactamase.[13] A low molecular weight gelator containing amino acid moieties confers liquid-crystalline (LC) gels.[14] Photo-sensitive spiropyran linking with dipeptide leads to supramolecular hydrogel to respond to both light and ligand–receptor interaction.[15] Despite these advancements, the application of supramolecular hydrogelator in controlled drug release has been less explored,[16] and there is even less exploration of supramolecular hydrogels for site-specific drug release. It is necessary and important to explore new ways

bxu@brandeis.edu.

Supporting Information Available: Synthesis of hydrogelator **1**, HPLC traces and LC-Mass data of the reduction of hydrogelator **1**, CD spectra and rheological data. This material is available free of charge via the Internet at http://pubs.acs.org.

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for creating supramolecular hydrogels as smart materials for controlled drug release at the specific sites or organs in a biological system.

Because colonic microflora secretes azo reductase to reduce the azo group into the corresponding amine, olsalazine, as a prodrug and a substrate of azo reductase, achieves colon specific drug delivery[17] via catalytic generation of mesalazine (or 5-aminosalicylic acid (5-ASA)) inside the colon at the site of inflammation.[18] This opportunity for reductive degradation of azo compounds by microflora of colon has led to the development of a score of polymeric azo compounds, which have found application for colon targeting since reduction and subsequent splitting of azo bond occurs only in the large instestine.[19] Encouraged by these results, we developed an olsalazine-containing supramolecular hydrogel as a candidate of smart biomaterials for the controlled-release. Specifically, we synthesized a hydrogelator (**1**) by using a tripeptide derivative that consists of a naphthyl group, two phenylalanines and one modified lysine residue carrying an olsalazine moiety in the side chain. **1** self-assembles to form a hydrogel at mildly acidic conditions. The reduction of olsalazine not only leads to gel-to-sol phase transition, but also releases 5-aminosalicylic acid. Via directly incorporating the prodrug into the nanofibers, this supramolecular hydrogel demonstrated a new way to encapsulate prodrug and to release the active ingredients. Because there is a large pool of prodrugs existing, this work contributes and benefits the future design of new smart biomaterials based on supramolecular chemistry[20] and prodrugs.

Figure 1 illustrates the structure of the hydrogelator (**1**), which contains a short peptide motif and an olsalazine moiety. We synthesized a small molecule hydrogelator **5**, which is a tripeptide derivative made by conjugating 2-(naphthalen-2-yl) acetic acid with Phe–Phe-Lys. In our recent study,[21] we found that the tripeptide derivative **5** forms a hydrogel at quite low critical gelation concentration (0.8 wt %). By conjugating **5** to olsalazine moiety through the epsilon amino group of the lysine residue, we expect that **1** will form a stable supramolecular hydrogel, which can act as a reservoir that, upon azo reduction, disassembles and releases the 5-aminosalicylic acid (5-ASA).

Scheme 1 shows the synthetic route of **1**. An HBTU activated compound **3** reacts with **5** to afford the hydrogelator **1** in 48% yields after the purification by flash column chromatograph. After obtaining **1**, we tested its ability to form a hydrogel in water by adjusting pH. Typically, 6.0 mg of **1** dissolves in 0.50 ml of water to give a clear solution, followed by changing pH to 5.0 to result in viscous suspension. Ultrasound sonication of the suspension for 2 min or increase of its temperature to ~60 °C followed by cooling to ambient temperature affords a transparent, yellow gel (Figure 2). This experiment demonstrates that **1** is an effective hydrogelator, which forms a stable gel in water at a concentration of 1.2 wt%. In order to further confirm that naphthyl group is necessary for compound **1** to form the hydrogel, we replaced the naphthyl group with an acetyl group. We found that the molecule acetyl-FFK-olsalazine (**7**) failed to form a hydrogel (Scheme S1 and Figure S5). While the hydrogelator L-**1** consists of L-phenylalanine and L-lysine, the hydrogelator D-**1** is made of D-phenylalanine and D-lysine.

In order to study reductant-mediated drug release from the hydrogel, we dissolved 11 mg sodium hydrosulfite in 0.2 ml of pH 5 buffer and injected the reductant over the hydrogel. The final concentration of hydrogelator **1** during reduction reaction is 0.86 wt%. After being incubated at 37 °C for 1 h, the hydrogel of L-**1** or D-**1** transforms into a light yellow suspension (Figure 2). HPLC and LC-Mass analysis of the suspension (Figure S1 and S2) confirm the conversion of **1** to the corresponding **2** (t_R = 18.2 min) and 5-aminosalicylic acid (t_R = 4.1 min). The identification of 5-aminosalicylic acid validates that this supramolecular

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hydrogel can act as a reservoir of prodrug and release the 5-aminosalicylic acid after reduction of the azo bonds.

Transmission electron microscopy (TEM) helps evaluate the extent of the self-assembly of the hydrogelator **1** during different stages of gel-sol transition. As shown in Figure 2, the hydrogelators L-**1** and D-**1** self-assemble to afford nanofibers with widths of 11 nm and 13 nm, respectively, and with lengths more than several microns (Figure 2C, G). In addition, the hydrogelator of D-**1** shows nanofibers with a right-handed helical structure (Figure S6). These nanofibers constitute the matrices (in the form of bundles or networks) of the hydrogels of **1**. The TEM images of the negative staining suspensions in Figure 2B and 2F indicate the loss of the long nanofibers after reductive cleavage of the azo bond, agreeing with that **2** fails to act as a hydrogelator. The dissociation of the three-dimensional networks of the nanofibers upon reduction indicates that the hydrogels of **1** should be able to release **5** upon the action of azo reducatase.[17]

Circular dichroism (CD) studies provide further molecular insight on the self-assembly of **1** and the gel-to-sol transition upon reduction. The hydrogelator L-**1** in the gel phase gives the CD spectrum with β-sheet signature as evident by negative bands at 218 nm and positive bands at 195 nm (Figure S3).[22] Upon reduction, the gel turns into the sol due to the conversion hydrogelator L-**1** to compound L-**2** and the release of 5-aminosalicylic acid. The CD signal of the β-sheet decreases significantly, indicating that L-**2** self-assembles less efficiently than hydrogelator L-**1** because of the loss of 5-aminosalicylic acid. The reduction of D-**1** generates D-**2** and also exhibits similar decrease of the signal between 190 nm and 204 nm, similar to the decrease of the signal of β sheets of the L-enantiomer (L-**1**) (Figure S3).[22] The hydrogel of D-**1** exhibits a strong CD band around 480 nm that is far from the chromophoric absorption region (ca. 360 nm) of olsalazine. This peak likely originates from a mesophase of D-**1**,[23] which agrees with the birefringence of the hydrogel of D-**1** (Figure S6).

We used oscillatory rheology to examine the viscoelastic properties of the hydrogels before and after reduction. Before the reductive cleavage of the azo bond, the hydrogels of L-**1** and D-**1** both exhibit elastic properties of a solid-like material, as demonstrated by the storage modulus (G') being almost an order of magnitude higher than the loss modulus (G") together with a weak frequency dependence of the elasticity (Figure 3). After the addition of the reductant, the values of the storage modulus (G') of the sample decrease nearly three orders of magnitude. The material behaves more like a viscous solution rather than an elastic gel. The obvious decrease of storage modulus (G') agrees with the gel-to-sol transition upon reduction reaction.

Because the site specific drug delivery also requires the supramolecular hydrogel to resist the attack of proteases *in vivo*, we synthesized the hydrogelator D-**1** to improve the stability of supramolecular hydrogels in biological environments. In order to evaluate its biostability, we incubated the hydrogel of D-**1** with proteinase K, a powerful enzyme that hydrolyzes a broad spectrum of peptides. The hydrogel of D-**1** remains unchanged (Figure 3) after incubated with proteinase K for 48 hours, indicating excellent biostability of D-**1** against proteinase K. That the addition of proteinase K fails to cause gel-to-sol transition of D-**1** also suggests that the hydrogel of **1** likely is insensitive to impurities.

In conclusion, we demonstrated that tripeptide derivatives conjugated with olsalazine exhibited excellent self-assembling properties to generate prodrug-containing supramolecular hydrogels and the reduction of the azo group can disrupt the supramolecular hydrogels and release the active ingredient. The use of D-peptides also should help preserve the stability of the hydrogels against proteases in upper gastro tract. Since it is easy to

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incorporate other therapeutics other than the prodrug in supramolecular hydrogels,[24] this work illustrates a new and facile way to use a prodrug with known metabolic pathways for generating supramolecular hydrogels as smart biomaterials for site-specific drug delivery.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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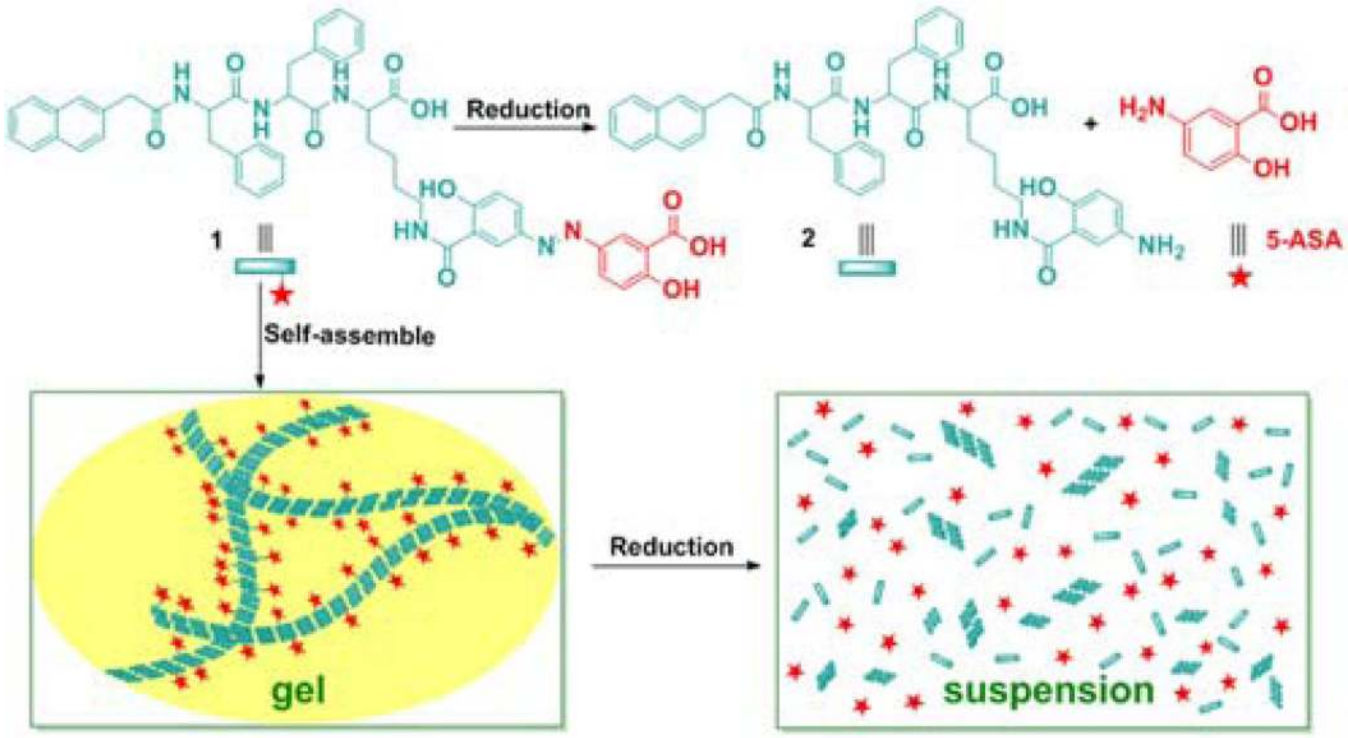

Figure 1.
Illustration of drug release from olsalazine-containing supramolecular hydrogel upon reduction.

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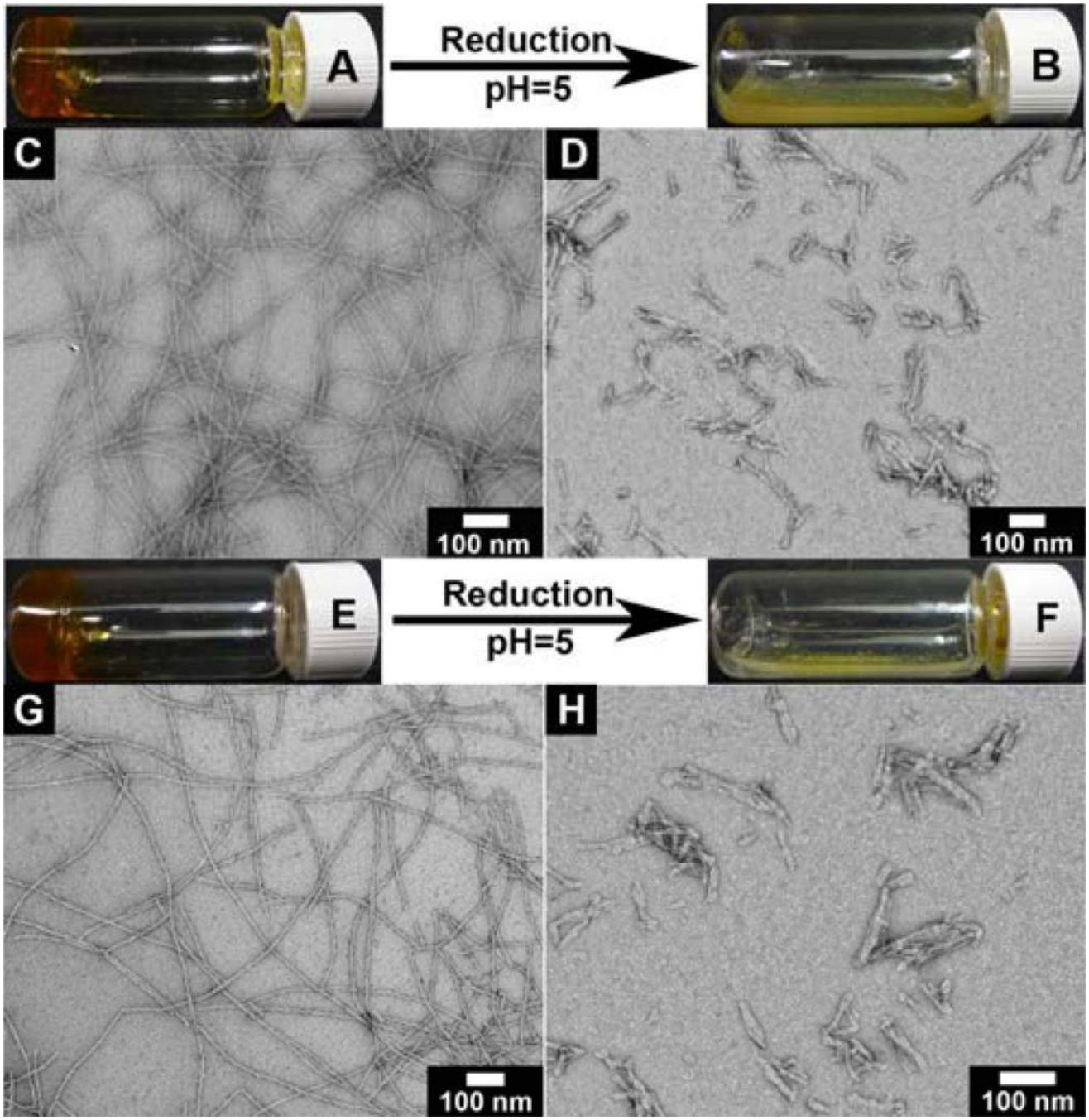

Figure 2.

Optical images of the hydrogels (A, E) of (A) L- **1** and (E) D-**1** (1.2 wt%, pH 5.0) and the suspensions (B, F) of (B) L- **1** and (F) D-**1** after reduction reaction on the hydrogels. Transmission electron micrograph (TEM) of the matrices (C, G) of the hydrogels of (C) L- **1** and (G) D-**1**; and the broken fibers (D, H) in the suspensions of (D) L-**2** and (H) D-**2**.

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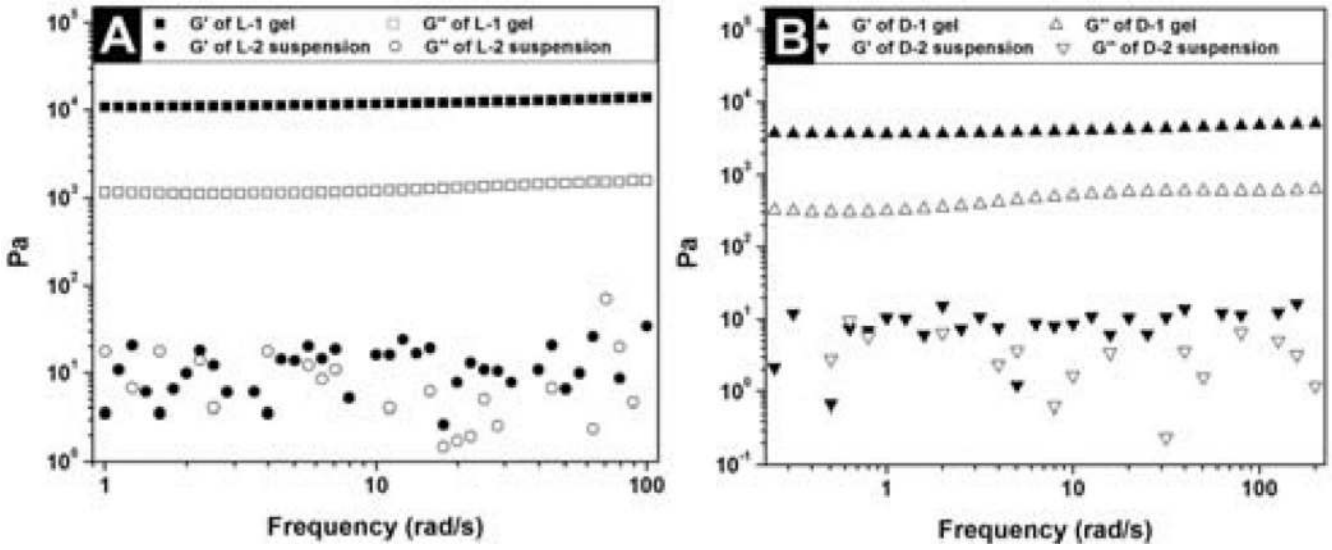

Figure 3.

(A) Frequency dependence of the dynamic storage moduli (G') and the loss moduli (G") of the hydrogel and suspension of L-**1** in; (B) frequency dependence of the dynamic storage moduli (G') and the loss moduli (G") of the hydrogel and suspension of D-**1**.

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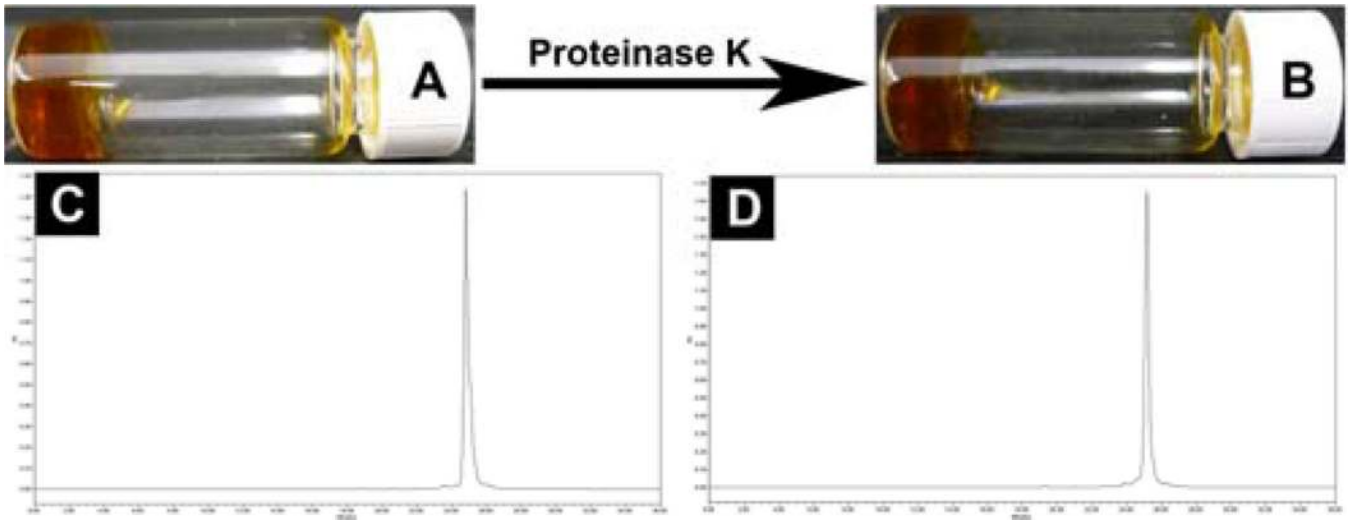
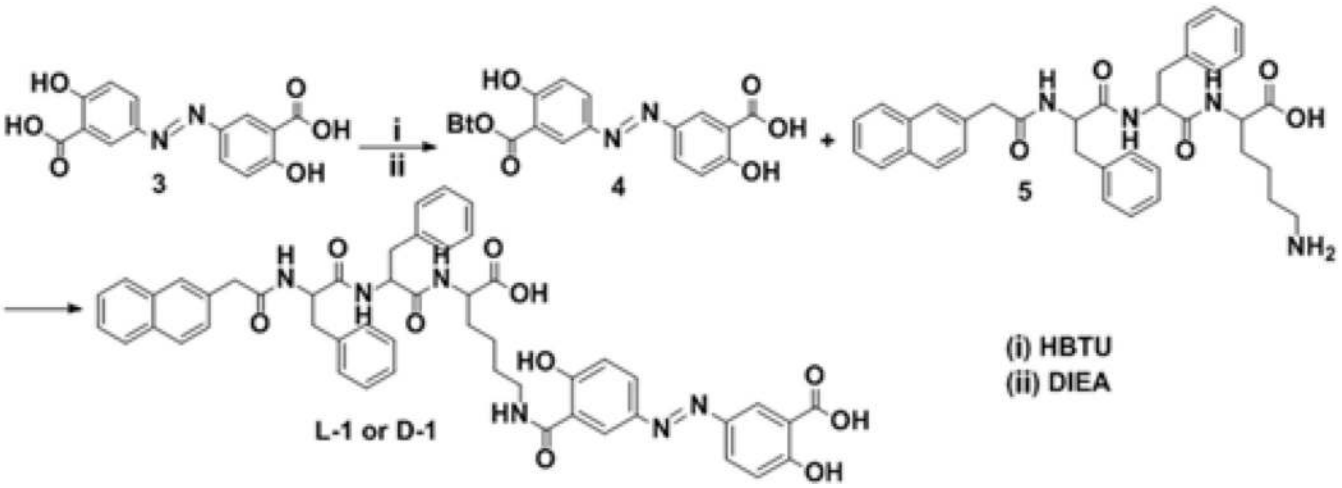


Figure 4.
Optical images of the hydrogel D-1 (A) before and (B) after proteinase K treatment. HPLC traces of the hydrogel D-1 (C) before and (D) after proteinase K treatment.



Scheme 1.
Synthetic scheme of the olsalazine-containing hydrogelator **1**.

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