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Versatile and Tunable Poly(Ethylene Glycol)-Based Hydrogels Crosslinked through the Ugi Reaction

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Abstract: The four-component Ugi condensation reaction has been investigated to assemble chemically crosslinked hydrogels using multivalent star-shaped poly(ethylene glycol) components. The resulting biocompatible hydrogels are highly versatile in composition and function. It is shown that acid, aldehyde, and cyanide components can be varied yielding materials with precise structure and tunable stiffness. Additionally, the resulting hydrogels were proven extremely robust to consecutive drying-swelling cycles. This property was explored to develop a reversible humidity colorimetric sensor gel. Overall, this work demonstrates the application of the four-component Ugi reaction as a powerful tool to quickly generate crosslinked gels with precise control in chemical composition.

Polymeric hydrogels are an important class of functional and tunable materials composed of a network of crosslinked hydrophilic polymeric chains.[1,2] Crosslinking provides hydrogels with mechanical strength, stability, and the ability to absorb water and biological fluids without losing structural integrity.[3,4] Hydrogels can be categorized based on the crosslinking mechanism, which can be either physical or chemical. Chemically crosslinked hydrogels are formed and maintained by covalent bonds established between polymeric chains, typically leading to higher mechanical strength and wide chemical diversity.[5] Due to their properties and the vast methods reported to obtain chemically crosslinked hydrogels, they have been used for a plethora of purposes such as gene delivery,[6] biosensing,[7] water purification,[8] and humidity sensing.[9]

The four-component Ugi reaction is a one-pot condensation reaction, first reported in 1959.[10] This multicomponent reaction involves an amine, a carboxylic acid, an aldehyde or a ketone, and an isocyanide, yielding peptidomimetic skeletons (Figure 1A).[11,12] It has been widely used in the pharmaceutical industry due to its potential to create products of broad structural diversity, generating large combinatorial libraries of drug compounds. More recently, it has also been explored to generate antibody-drug conjugates.[13,14] Within the field of materials science, the four-component Ugi reaction has been used to generate multifunctional polymers[15] or to crosslink natural polymers (hyaluronic acid,[16,17] alginate,[18] and pectin[19,20]) yielding hydrogels, or a mixture of natural and synthetic polymers,[21] yielding microgels in microfluidics systems. To expand the application of the four-component Ugi reaction in materials science, we report chemically crosslinked hydrogels using poly(ethylene glycol) (PEG) multivalent components. PEG is a very attractive synthetic polymer since it is biocompatible, non-ionic, and offers the possibility to vary the molecular size and multivalency, as well as chemical functionalities at the terminal groups.[4] The chemical versatility of our PEG-based hydrogels is introduced in a facile, controlled, and precise manner by varying the Ugi reaction starting components, thus allowing the creation of hydrogels with different structures, mechanical properties, and functions. Chemically crosslinked 4-arm PEG-based hydrogels[22] have been previously reported using two-component systems and click chemistry reactions, however without the ample versatility provided by the four-component Ugi reaction as a crosslinking method. Polymers and hydrogels, due to their availability, high flexibility, high water permeability, and low weight are attractive materials to fabricate low-cost humidity sensors.[23,24] Colorimetric sensors based on cobalt chloride are among the most commonly studied.[23] However, due to cobalt chloride toxicity,[25] there are benefits in its immobilization, as typically used in fiber-based colorimetric sensors.[26,27] However, in these cases, the potential to re-use the support and the cobalt chloride or to properly dispose of cobalt chloride is not possible. Due to the excellent reversible drying-swelling capabilities of our Ugi-based hydrogels, we show-cased its potential application as a humidity sensor where both hydrogel and cobalt chloride can be re-used or separately and appropriately disposed of in the end.

The four starting components to form PEG-hydrogels via the Ugi reaction (UPH) were: (i) free amine groups present in commercial 20kDa star-shaped PEG-$[NH_2]_4$; (ii) propionaldehyde; (iii) free carboxylic acid groups present in functionalized 20kDa star-shaped PEG-$[COOH]_4$; (iv) isopropyl isocyanide (for general procedure and characterization, see Supporting Information). It is widely accepted that the reaction conditions influence the establishment of the Ugi reaction.[28] Here, the reaction temperature, a molar excess of aldehyde and isocyanide, solvent,

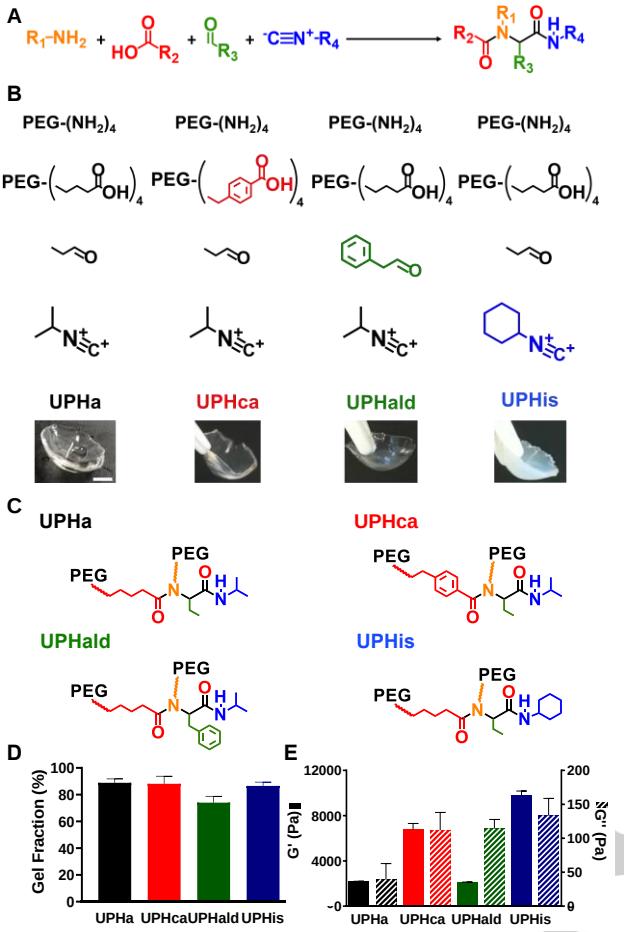

Figure 1. PEG-based hydrogels crosslinked via the four-component Ugi reaction provide a platform for chemical diversity and tunable mechanical properties. A) Schematics of the four-component Ugi reaction B) Components used to form the hydrogels and representative images of the obtained hydrogels. Scale bar is 0.5 cm. C) Schematic representation of the crosslinking moieties generated by the four-component Ugi reaction upon variation of each reaction component D) Gel fraction of the different hydrogels. E) G' and G'' of the different hydrogels formed.

pH (when appropriate) and 4-arm PEG concentration were varied to determine the best parameters for hydrogel formation (Figure S1). The time taken for hydrogel formation, as well as hydrogel's rheological properties were monitored to determine the most suitable conditions to form mechanically stiff hydrogels.

The Ugi reaction is typically conducted at either room temperature or 60 °C, near the ebullition temperature of methanol.[28] For our system, gelation only occurred at 60 °C. It is known that at higher temperatures, the reaction yield is increased granting faster connectivity of the network.[18,29]

The aldehyde and the isocyanide are very important components for the Ugi reaction. The poor choice of the aldehyde can lead to a low concentration of the iminium ion, which is critical to yield the Ugi scaffold because, without the nucleophilic attack of the isocyanide to the iminium ion, the scaffold is not formed.[28]

To ensure these steps were completed, two reactions were attempted with 10 molar (UPHa) and 1 molar (UPHb) excess of aldehyde and isocyanide in relation to the amines in the system (for amplitude and frequency sweep assays, see Figure S5 and

Table 1. Gel fraction, G' and G'' of the hydrogels formed.

Hydrogel	Gel fraction [%]	G' [Pa]	G'' [Pa]
UPHa	90±3	2156±98	40±21
UPHb	93±3	5015±70	78±17
UPHc	93±4	1520±98	37±5
UPHd	74±4	2120±54	56±33
UPHca	88±6	6800±112	112±26
UPHald	74±5	2112±16	114±13
UPHis	86±3	9794±382	170±47

Figure S6, respectively). In both cases, there was hydrogel formation. The storage modulus (G') of both hydrogels is within the same order of magnitude (Table 1, Figure S1), despite being 2.3-fold higher for UPHb. Furthermore, the distance between the G' and the loss modulus (G'') (over one order of magnitude), combined with a lack of dependence of the G' toward the frequency, are indications of mechanically structured hydrogels.[30] UPHa was formed in 5 hours instead of 24 hours as observed for UPHb (Figure S1) because high concentrations of aldehyde and isocyanide increase the kinetics of the Ugi reaction.[28] As such, 10 molar excess of aldehyde and isocyanide (relative to the density of amine groups) were selected to proceed with further studies.

Typically, the Ugi reaction is performed in methanol as it is a protic solvent that stabilizes the polar derivatives of the Ugi reaction intermediates.[31] Also, in opposition to the reaction products, the initial reagents are usually very soluble in this solvent.[28] However, in some reports, water was used as a solvent.[32] In an attempt to work in less toxic conditions, the reaction was performed in water. Considering that the pKa of butyric acid (functionalized on the PEG-$[COOH]_4$ component), the pH was lowered to 2-3 to ensure that the carboxylic acid component was protonated. No gel formed under these conditions. The solvent was changed to a mixture of methanol and water with a ratio of 1:1. As it is described in the literature,[33] the addition of a protic solvent such as methanol can be crucial to the Mumm rearrangement because the formation and stability of the final product demand a H-transfer from the solvent to the product structure. The hydrogel UHPc made in water:MeOH 1:1 formed after 24 hours (Figure S1) and its G' is very similar to the hydrogel formed in methanol only (UPHa).

Finally, the concentration of PEG-$[NH_2]_4$ and PEG-$[COOH]_4$ was varied between 1% and 10% (w/v), namely 1%, 2%, 5% and 10%. The reaction was unsuccessful when the concentration of PEG-$[NH_2]_4$ and PEG-$[COOH]_4$ was 1% and 2%, indicating that for a network to be formed with a 4-arm PEG, the minimum concentration of the PEG components was 5% (UHPd) (Table 1, Figure S1).

The formation of PEG-based hydrogels crosslinked via the Ugi reaction was confirmed by the negative control formulations that did not gelate, each missing one of the four components, proving the need of the amine, carboxylic acid, aldehyde, and isocyanide to successfully yield the Ugi product and subsequent hydrogel formation (Fig S2). ATR-FTIR also showed the formation of an amide bond at 1600 cm^{-1} attributable to C=O stretching (Fig S3).

In conclusion, for faster gelation (5 hours), the preferred conditions for hydrogel formation were: reaction temperature of 60 °C; 10 molar excess of both aldehyde and isopropyl isocyanide concerning the amines present in the system; the use of methanol as the solvent; concentration of 10% (w/v) of PEG-$[NH_2]_4$ and

PEG-[COOH]$_4$ (Figure S1). In previous works reported in the literature regarding hydrogel formation via the four-component Ugi reaction, the reaction was carried in similar conditions apart from the temperature and the solvent.[14,19,34] Overall, the hydrogels obtained in this work are mechanically stiffer.[18]

The nature of the components used in the Ugi reaction will affect the diversity of the crosslinking and, consequently, the chemical diversity and properties of the final hydrogels (Figure 1B, Figure 1C). Since the aminated 4-arm PEG molecule was commercially available, only the carboxylic acid, the aldehyde, and the isocyanide were varied. In each new hydrogel formulation, every component was introduced in the same concentration as in UPHa. Control experiments were also performed where one of the four components of the new hydrogels was removed (Figure S2), further proving the significance of each component to the success of hydrogel formation. To vary the carboxylic acid component, 4-(aminomethyl)benzoic acid was successfully functionalized into 4-arm PEG molecules. The introduction of this component led to UPHca gels, with a very similar gel fraction in comparison to UPHa (Figure 1D, Table 1) but 3.2 times stiffer (Figure 1E; Table 1, Figure S6). The UPHald hydrogel resulted from the substitution of propionaldehyde for phenylacetaldehyde and the lower gel fraction in comparison to the UPHa (Figure 1E, Table 1) indicates that less polymer reacted to form the network, which may be related to steric hindrance effects during the reaction due to the bulky benzene ring. Additionally, when a cyclic isocyanide (cyclohexyl isocyanide) was replaced by isopropyl isocyanide (UPHis), hydrogels 4.5 times stiffer were formed (Figure 1E, Table 1) despite the similar gel fraction to UPHa. The increased stiffness can be explained by the conformational flexibility of the cyclohexyl ring[35] which can induce a more efficient packing of the flexible 4-arm PEG molecules resulting in an overall compact structure. Since reversible swelling in response to external stimuli is one of the most important properties of a hydrogel[36], the possibility to shrink and swell UPHa hydrogels upon drying at 60°C was judged.

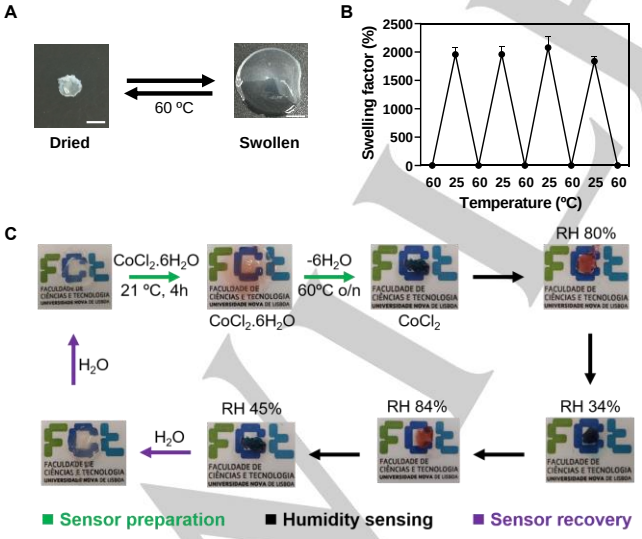

Figure 2. Hydrogel reversibility to dry and humid conditions and proposed humidity sensor. A) Drying-swelling cycles. Scale bar is 0.5 cm. B) Swelling factor of UPHa through the cycles. C) UPHa as a colorimetric humidity sensor. Adsorption of $CoCl_2$ to the network by solvent exchange. The hydrogel was dried overnight at 60°C and changed its color. Varying the humidity at which the hydrogel was exposed, $CoCl_2$ changes its hydration state, visible at the naked eye by color change. RH – Relative humidity. The hydrogel can be recovered by a reversible process through a new solvent exchange.

After drying, UPHa was swollen in water for 10 minutes (Figure 2A). UPHa hydrogel's network was tested for four cycles by monitoring the swollen hydrogels' mass (Figure S4) and swelling fraction at room temperature (Figure 2B). Taking advantage of the reversible drying and swelling behavior, a reversible colorimetric humidity sensor was designed. The adsorption and desorption of chemical and biological molecules from the hydrogel structure can be performed by a simple solvent exchange (Figure S8 and Figure S9). A dry hydrogel was incubated with cobalt chloride hexahydrate ($CoCl_2.6H_2O$) solution. Cobalt chloride is known for its color changes when in contact with water,[26] as cobalt is a transition metal capable of forming stable, colored metal complexes. The anhydrous, blue-colored cobalt (II) chloride changes its color towards complexation with water, forming the hydrated, pink-colored cobalt hexahydrate (II). The reverse process can be achieved by drying through thermal treatment. The adsorption of $CoCl_2.6H_2O$ molecules onto the swollen UPHa hydrogel was visible by naked eye due to the pink-colored hydrogel obtained. The cobalt hydrogel was dehydrated overnight resulting in a blue coloration (Figure 2C). Its capabilities as colorimetric humidity sensors were further explored by placing the cobalt hydrogel in a controlled humidity cabinet. When exposed to 80-84% humidity, the hydrogels turned pink while, when submitted to lower humidity values (36-45%), the gels turned blue. This process is reversible for at least 10 cycles (Figure S9). A 2% (w/w) difference of the hydrogel mass was observed between the humid (80-84%) and dry state (34-45%). Also, the initial hydrogel can be recovered by performing a new solvent exchange against water (Figure 2C, Figure S10, Table S1).

Finally, to explore different applications of the poly(ethylene glycol)-based hydrogels crosslinked through the Ugi reaction, we verified the biocompatibility of the obtained hydrogels with the tested isocyanides in UPHa and UPHis. Biocompatibility was assessed by indirect and direct assays (Figure S11). In the indirect assay, it is assessed if the materials leak any toxic by-products into the medium by incubating fibroblasts with medium supernatants. The cells' viability was evaluated by a MTT assay.[37] Fibroblasts cultured with the medium from the UPHa and UPHis hydrogel showed high cell viability (UPHa 95.5±4.7% p-value <.0006; UPHis 90.7±8.5% viability, p-value <.0014) in comparison with the positive control (100±13% viability). In contrast, the fibroblasts that were incubated with a latex glove (negative control)[38] presented low cell viability (16.1±7.6%, p-value <.0024). The cells' viability shows no significant difference in comparison to the positive control. Therefore, any excess of cyanide and methanol was either evaporated in the gelation process or was completely removed during hydrogel washing and sterilization. A two-tailed independent sample t-test was used for statistical analysis. In the direct assay, the fibroblasts in contact with latex died in its proximity adopting a more rounded morphology. In opposition, the cells in contact with UPHa and UPHis grew in a compact monolayer in their typical elongated shape.

In conclusion, we report, for the first time, the formation of biocompatible chemically crosslinked PEG-based hydrogels via the four-component Ugi reaction, where functionalized 4-arm star-shaped PEG molecules were used as the amine and acid components. Varying the starting components, it is possible to produce hydrogels with precise chemical modifications and different mechanical behaviors in a defined and controlled manner. These hydrogels can be reversibly dried and rehydrated

and were assessed as sustainable alternatives for colorimetric humidity sensors, where both the support (hydrogel) and cobalt chloride can be re-used or separately disposed of in the end. Future studies on hydrogel formation with different Ugi reaction components and 4-arm star-shaped PEG molecules with different arm-lengths will allow a deeper adjustment of the hydrogels' properties, opening the possibility to be used in different settings from biomedical to technological applications.

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Keywords: crosslinking • gels • poly(ethylene glycol) • sensors • Ugi reaction

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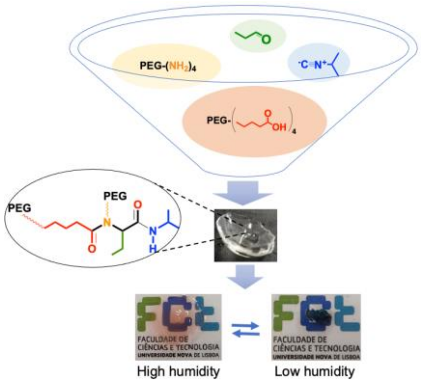

Multivalent PEG-based hydrogels as potential sensors: The four-component Ugi reaction allows the fast and controllable crosslinking of multivalent PEG polymers. Using diverse acid, aldehyde, and cyanide components, biocompatible and versatile hydrogels with different stiffness were formed. Due to its ability to endure successive dry-swelling cycles, these gels can be used as humidity sensors (see picture).

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