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ARTICLE TYPE

Dissolvable Metalhydrogels for Controlled Release: Evidence of a Kinetic Supramolecular Gel Phase Intermediate

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Two metalhydrogels based on an amino acid-based ligand and Zn(II) salts were synthesized. These hydrogels show an uncommon, reversible, time-dependent transformation from opaque to transparent state. These hydrogels also exhibit gradual dissolution in water (pH ≤ 7) over time. A water-soluble stimulant, caffeine, could be *in-situ* loaded into the hydrogels and slowly released during dissolution.

Self-assembled supramolecular hydrogels have attracted much attention during the past decades because of promising high-tech applications in very diverse fields such as biomedicine, nanoelectronics and catalysis.¹ These colloidal materials can be formed either by low- or high-molecular-weight hydrogelators by means of non-covalent interactions (e.g., π - π stacking, Van der Waals forces, hydrogen bonds, hydrophobic interactions).² Within these materials, metallohydrogels³ represent an important subclass where at least one metallic element has been incorporated by some means into the gel network.

Herein, we report two unique, amino acid based LMW metalhydrogels (*viz.* ZNVA and ZPVA; Figure 1), which displayed an unprecedented (*opaque gel*)-to-(*transparent gel*) transition for this type of gels. Moreover, the equilibrated gels showed a remarkable time-dependent dissolution in both water and acidic aqueous solutions (pH ≤ 7), which could be used for the controlled release of entrapped molecules. Figure 1 exhibits the spontaneous formation of hydrogels, namely ZNVA and ZPVA, upon mixing of two aqueous solutions containing amino acid (L-valine) based ligand [i.e., L-3-methyl-2-(pyridine-4-yl-methylamino)-butanoic acid] with sodium acetate as ligand systems (VA) and Zn(II)-based salts [i.e., zinc nitrate hexahydrate = ZN and zinc perchlorate hexahydrate = ZP], respectively. These ligands were easily synthesized by minor modification of a previously reported procedure⁵ (Section S1). These hydrogels adopt an opaque white color immediately after formation but loses their opacity completely over time becoming transparent after standing one week at RT without formation of solution phase intermediate. To the best of our knowledge, the changes in the optical properties of gel-based materials reported so far involve either temperature-triggered transitions⁴ or translucent/transparent-to-opaque transitions as a consequence of domain aggregation to sizes that start to scatter light (e.g., via crosslinking). However, in our case, the *gel-to-gel* transition occurs naturally from an opaque phase to a transparent phase.

The transparent gel could be transformed into the original opaque gel upon a heating/cooling treatment through the corresponding solution phase. The so-obtained opaque gel showed the same thermal, morphological and mechanical properties than the original opaque gel obtained at RT, and hence it also underwent evolution towards the transparent material upon ageing. The entire cycle could be repeated multiple times without any appreciable difference in the obtained materials after each cycle. This phenomenon indicates a reversible change in the structure of the supramolecular aggregate in the presence of water.

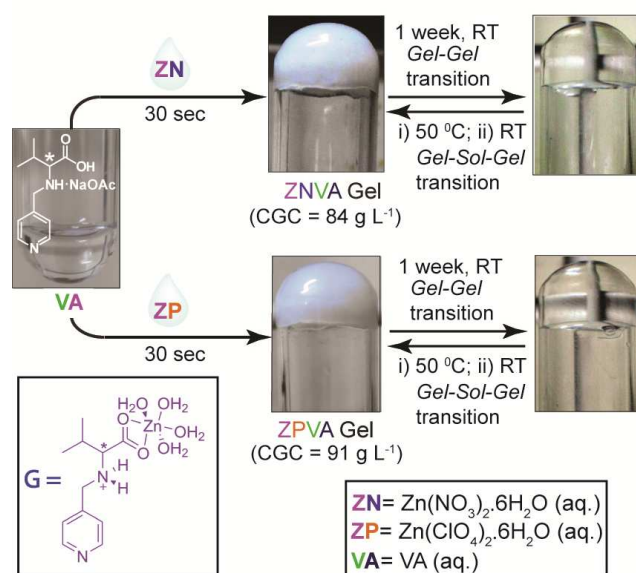


Figure 1. Synthesis of ZNVA and ZPVA hydrogels at CGC. Total volume of each solution = 0.5 mL. Inset: Plausible structure of the fundamental unit (G) of the gel fiber deduced from MALDI-TOF spectroscopy.

These two hydrogels are similar to previously reported ZAVP hydrogel^{5a} where the metal ion precursor was zinc acetate (ZA) and (pyridine-4-yl-methylamino)-butanoic acid) with sodium perchlorate (VP) was used as ligand system. ZAVP hydrogel exhibited self-healing and multiresponsive nature but it was difficult to dissolve it in water under ambient conditions. The critical gelation concentration (CGC) for opaque ZNVA and ZPVA hydrogel was established in 84 g L⁻¹ and 91 g L⁻¹, respectively. The hydrogels were thermoreversible and the *gel-to-sol* transition temperatures (T_{gel}) were 49 ± 1 °C and 58 ± 1 °C for

ZNVA and ZPVA, respectively. However, the transparent ZNVA and ZPVA exhibit a lower *gel-to-sol* transition temperature (41 ± 1 °C and 45 ± 1 °C, respectively). Complete *sol-to-gel* transitions occurred while cooling the corresponding isotropic solutions for 15 minutes. Interestingly, the results obtained from a large screening of metal ions established that the gelation was specific in the presence of Zn(II) salts as coordinating metal. The contribution of the counter-anion was, however, less critical and similar gel could be obtained by using acetate instead of nitrate and perchlorate. In this communication, we focus on the detailed study of the ZNVA and ZPVA as model systems.

Electron microscopy imaging of the corresponding xerogels confirmed the presence of nanostructured networks in each case with apparent differences in the crosslink densities (Figure 2). The viscoelastic nature of the hydrogels was confirmed by oscillatory rheological measurements (Figure 2 and Figure S1). The average storage modulus ($G' > 10^5$ Pa) of as-synthesized gels (6 h after synthesis) was one order of magnitude higher than the loss modulus (G'') and constant over the entire range of frequencies (0.1-10 Hz) (Figure S2). Reproducible and relatively constant dissipation factors ($\tan \delta = G''/G' \approx 0.1$) during DFS indicated a good tolerance of the hydrogels to external forces. Further dynamic time sweep (DTS) measurements at RT (Figure S2) at 0.1% strain and 1 Hz frequency confirmed the temporal stability of the hydrogels. These values of mechanical properties remained intact up to 4 days of synthesis of the hydrogels. But, surprisingly, mechanical properties of both the hydrogels deteriorated as they became transparent with time. Storage modulus of both of these gels decreased

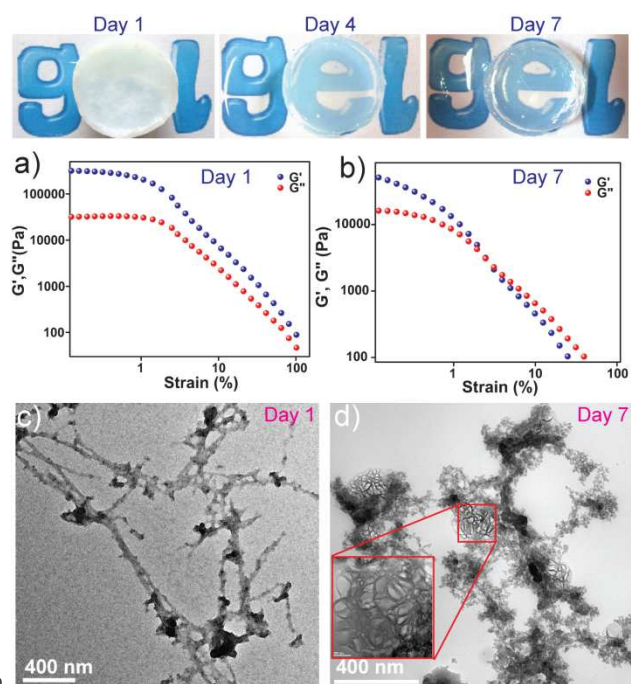


Figure 2. Top: Opaque to transparent conversion of ZNVA hydrogel with time. Middle: Dynamic strain sweep (DSS) plot of a) opaque and b) transparent ZNVA hydrogel. Bottom: TEM image of (c) opaque and (d) transparent ZNVA hydrogels.

(10^4 Pa $< G' < 10^5$ Pa) with insignificant change in viscous moduli. Brittle nature of the transparent hydrogels were attributed to their early fracture at strain (deformation) below 10%

compared to their opaque counterparts ($> 10\%$) (Figure 2). Hence, oscillatory rheological measurements suggest that transparent gel has a lower mechanical strength than the precursor opaque gel. TEM images (Figure 2) obtained from opaque and transparent gel materials also support the hypothesis based on the rheological measurements. Initial fibrillar supramolecular aggregates degrade to smaller and thinner aggregates with time as the gel goes to transparent phase from opaque phase. So, the achievement of a stable transparent gel over time indicates the formation of new aggregates that are shorter than the visible wavelength region, which could be in good agreement with the observed decrease in the storage modulus of the material, albeit the overall network may be more ordered.

Contrasting thermal stability (Figure S3) of the corresponding xerogels also support the gradual weakening of the hydrogels during their journey from opaque to transparent state. Similar pattern of the plots indicates the basic structure of the gel network remains similar in both opaque and transparent state which can also be explained by the PXRD and the IR spectra (Figure S4 and S5). But deviation in the patterns (Figure S3) arouse $\sim 110^\circ\text{C}$ indicates a subtle loss of mass from the gelator self-assembly which eventually reflects in a considerable difference in leftover mass ($\sim 6\text{-}7\%$) at the end of the experiment (800°C).

ZNVA and ZPVA have also shown rapid responses towards various stimuli (Figure S6) (e.g., thermal, mechanical, pH), which clearly illustrate the dynamic non-covalent and self-assembled nature of the gel network. These two hydrogels showed reversible *sol-to-gel* transition in the presence of acid (TFA) and base (ammonia). The gels turned into a clear solution phase in the presence of TFA due to disruption of the H-bonding network⁶ (Figure S6). Addition of ammonia quenched the excess acid, which eventually allowed the restoration of the H-bonding and consequently the gel phase. Moreover, addition of ammonia could also solubilize the gel due to the formation of water soluble zinc tetrammine complex. Addition of TFA neutralized the ammonia from the metal centre liberating the metal ion for complexation, which led to the recovery of the gel phase. This phenomenon suggests the presence of the Zn(II)-complex unit, which self-assembles into individual gel fibers (Figure 1).

Furthermore, we found that the xerogels obtained from the corresponding ZPVA and ZNVA hydrogels, could be easily restored as the original hydrogels upon exposition to the same amount of water that the hydrogels ensnared (Figure 3). ZNVA and ZPVA were also found to be dissolved in excess of water (i.e., 1.5 times of their volume at the CGC; pH = 7) yielding a colloidal solution within 6-7 h at RT. Although it is expected that the dilution of physical gels prepared at the CGC may cause the collapse of the supramolecular network, the *gel-to-sol* transition by simple dilution was in this case exceptionally reliable and fully reversible. Thus, the gel phase could be restored upon evaporation of the same amount of water required to dissolve the gel (Figure 3).

This kind of time-dependent and efficient solubility of hydrogels in neutral as well as acidic pH motivated us to study the encapsulation and simultaneous degradation-release behavior of these materials using caffeine as a model drug.

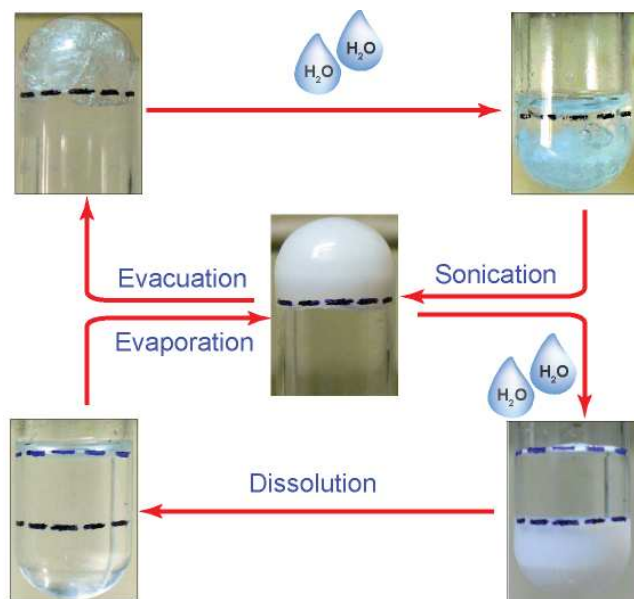


Figure 3. ZNVA and ZPVA hydrogels show *gel-to-sol* and *gel-to-xerogel* reversible transitions.

Although caffeine is a small polar compound, it is hydrophobic enough to pass through biological membranes. Hence, it has been used in numerous studies as a model compound to investigate the ability of hydrogel materials as drug delivery vehicles.⁷ In our study, we employed 2 mL gel blocks having a caffeine concentration of 0.15 g L^{-1} . These blocks were immersed in 4 mL citrate buffer (pH = 4.74) and 4 mL deionized water (pH = 7) separately. The dissolution of the gel, as well as the subsequent cumulative drug release was monitored over time. As shown in Figure 4, the caffeine loaded ZNVA showed a sustained release until it got completely dissolved within 5 h in citrate buffer and 6 h in water (Figure 4).

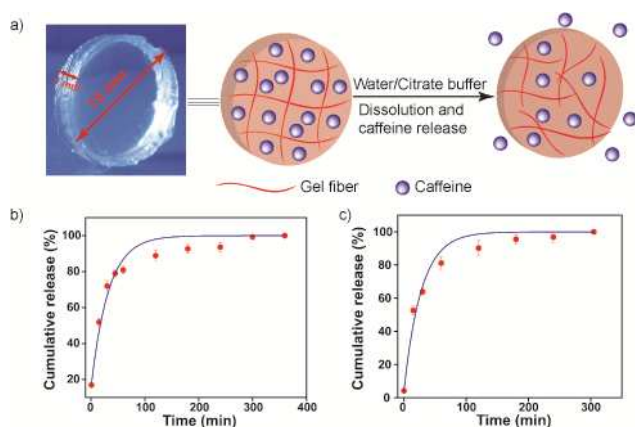


Figure 4. a) Self-standing caffeine-loaded hydrogel and caffeine release during dissolution of the gel matrix. b)-c) Cumulative release profile in b) citrate buffer and c) water.

We used MALDI-TOF analysis to get insights into the structure of the gelator and the mechanism of the dissolution of the hydrogels. Detailed study of the obtained spectra suggested that the metal complex (Figure 1 and Figure S7 and S8; $m/z = 345$) formed due to the replacement of two water molecules of Zn(II) hexaquo complex (from the precursor metal salts; zinc

nitrate hexahydrate and zinc perchlorate hexahydrate) by ligand V during gelation, is probably the fundamental unit of the gel fiber G (Figure 1 and Figure S7). The water soluble nature of ZPVA and ZNVA could be explained as Zn (II) co-ordinated to 4 water molecules in G. In contrast, the fundamental unit of our previously reported hydrogel ZAVP⁵ was more hydrophobic in nature (i.e., Zn(II) was connected to two water molecules, one acetate anion and one molecule of ligand V) which makes it more difficult to get dissolved in water by further solvation of the aggregates. Hence, the inherent hydrophilicity and hydrophobicity of the gelator constitutes one of the key factors that govern the solubility of a bulk hydrogel in water. This also explains the deterioration of gel strength during the course of transformation of the hydrogels from opaque to transparent state. The hydrophilic nature of the gelator (G) makes it remain in equilibrium with its components (zinc (II) hexaquo complex and the ligand V) in presence water. Hence, the gradual hydrolysis of the gelator in the self-assembly weakens the fibers and gives rise to transparent hydrogels of poor mechanical strength. The transparent hydrogels can go back to their native opaque state through *sol-gel* transition by heating. This implies that the sol state is equivalent to the solution after mixing of precursor before the original gel formation.

Overall, these results suggest that the initially formed supramolecular aggregates (opaque gel) is a transient kinetic product, while the final transparent gel likely correspond to a lower energy level, which could be the thermodynamic minimum as no evidences of subsequent crystallization have been observed after several months. As far as we are aware, this is the first time that a kinetic supramolecular metallohydrogel is isolated and its transition to a lower energy transparent gel phase recorded. Although the formation of such kinetic aggregates may occur in other supramolecular gels, the permanent opacity of the bulk material and/or the short lifetime of the aggregates have probably prevented the identification of this intermediate gel phase in other cases.

In summary, we have shown the facile synthesis of two novel non-polymeric supramolecular metallohydrogels by combining an amino acid-based ligand (i.e., L-3-methyl-2-(pyridine-4-yl-methylamino)-butanoic acid) and Zn(II) salts. These hydrogels displayed unique reversible opaque to transparent phase transition at room temperature, multiresponsive and reversible *gel-to-sol* and *xerogel-to-gel* transitions, as well as gradual dissolution in aqueous medium within a wide range of pH at RT. This smooth transition was employed for the *in vitro* release of a model polar drug at both neutral and acidic pH. The unprecedented (*opaque gel*)-to-(*transparent gel*) transition evidences the formation of a kinetic supramolecular gel that undergoes self-evolution towards a different gel network of lower energy. The isolation of this intermediate gel phase may have important mechanistic implications on the theory of gelation. Further analyses are ongoing in our laboratory to quantitatively interpret the mechanism of this transformation.

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