

Anion-triggered melamine based self-assembly and hydrogel†

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We found that melamine in its protonated form could be triggered by oxoanions such as NO₃⁻, PO₄³⁻, ATP, and SO₄²⁻ to form superstructures and to gelate a large amount of water molecules, presumably resulting from the electrostatic and hydrogen bonding interactions operating in a synergistic manner; the gelation can be reversibly switched off/on by increasing/decreasing pH or temperature.

Although melamine (M) has since the late 1930s been widely used as a raw material in the chemical industry, not until 2008 did it become a notorious molecule in the scandal of illegally adding M to dairy products to falsely increase protein content. Such conscienceless behavior has led to serious kidney damage in tens of thousands of infants and even several cases of death.¹ The damaging effect is considered to likely result from the aggregates of M with associated impurities or metabolites existing in body fluids such as cyanuric acid (CA) and uric acid (UA) bearing an imide moiety.² These aggregates may further associate into clusters that stay in the renal tubules, leading to renal failure or kidney stones. Since UA concentration in serum and excretion of infants is generally higher than that of older age groups on the basis of body weight,³ the damaging effect of M on older age groups is considered to likely be less serious than that on infants. Meanwhile, melamine is also known to be an excellent building block for constructing elegant functional supramolecular self-assemblies because of its rigid and planar structure together with multiple hydrogen bonding and π - π stacking capacity.⁴ For example, a rosette pattern has been observed in M-CA or M-UA assemblies that are stabilized by complementary NH...O and NH...N hydrogen bonding.⁵ In some cases, M or its derivatives could form supramolecular hydrogels⁶ or organogels,⁷ a class of robust functional soft materials that have received considerable recent attention,⁸ integrating one or several imide components by triple hydrogen bondings. Those reports, however, focus on neutral M that forms superstructures *via* hydrogen bonding interactions with other species. On the basis of the structure of M (Fig. 1a), the original hydrogen bonding accepting sites would be changed to excellent hydrogen bonding donating sites if the N atoms in the triazine ring are protonated ($pK_a = 5.10$).⁹ Oxoanions in a good conformation may act as “bridges” to form novel superstructures with the protonated M.

Therefore, it appears to be an important issue whether some biologically related anions are capable of triggering protonated M to self-assemble into superstructures and even to gelate water molecules. If this could be confirmed, the potential health risk of M for older age groups should be paid more attention or even be reevaluated. Supramolecular chemistry including gelation of protonated melamine is thus believed to be a challenging subject that deserves to be explored. Here we wish to report our findings that several oxoanions can indeed trigger protonated M to form 1-D nano-/microscale fibers or sheets and even supramolecular hydrogels under slightly acidic conditions (Fig. 1a). The results are expected to shed light on the future medical investigations on M-related diseases and promote the development of protonated melamine supramolecular chemistry.

Considering its biological significance, PO₄³⁻ was first chosen to test the possibility of triggering protonated M to aggregate into superstructures and to form supramolecular hydrogels. It was found that in neutral or alkaline aqueous solution even at a high total concentration of 700 mM, 6 equivalents of PO₄³⁻ mixed with 1 equivalent of M did not lead to any observable change. However, it was unexpectedly observed that a stable and opaque gel was obtained at room temperature when the solution pH was adjusted with dilute HCl solution to 2–5. A clear solution could be regenerated when the pH was increased to 7 with dilute NaOH. This process could be reversibly repeated for many times by alternatively increasing and decreasing the pH (Fig. 1b). Raising the temperature over 50 °C also led to a similar gel-to-sol state transition of the supramolecular hydrogel, while the gelation

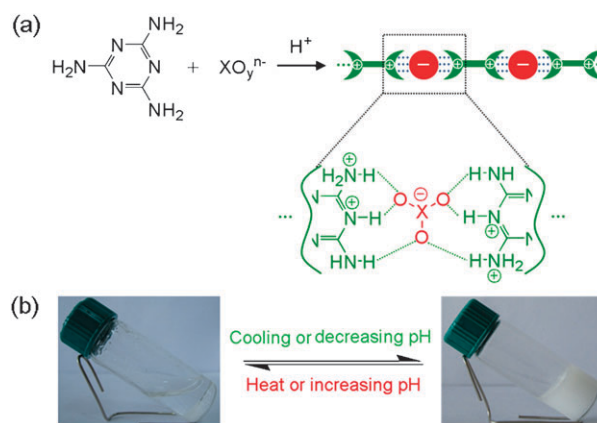


Fig. 1 (a) Schematic illustration of protonated melamine self-assembly and hydrogel triggered by oxoanions. (b) Reversible gel-to-sol and sol-to-gel state transitions of $M(H^+)_m-XO_y^{n-}$ supramolecular hydrogel induced by temperature and pH, respectively. Note that $M(H^+)_m$ represents protonated M and XO_y^{n-} represents PO₄³⁻, NO₃⁻, SO₄²⁻, or ATP.

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could be re-induced upon cooling. Such a reversible process could be repeated infinitely without any observable loss of its gelation capacity (Fig. 1b). The system could exist in a partial gel state even at a molar ratio of M to PO_4^{3-} down to 1:3, whereas in the absence of PO_4^{3-} no gelation could be observed at any accessible concentration of M in acidic aqueous solution. The xerogel from a freeze-drying procedure was shown by field emission scanning electron microscopy (FESEM) to possess well-defined three-dimensional networks composed of micro-/nanoscale fibers that could provide a good matrix for gelating water molecules (Fig. 2a). These uniform micro-/nanoscale structures were also confirmed by transmission electron microscopy (TEM) images (Fig. S1 in ESI†).

Surprisingly, we found that other oxoanions such as NO_3^- , SO_4^{2-} , and even the bulky anion adenosine 5'-triphosphate (ATP), were also able to induce protonated M to form supramolecular hydrogels (Fig. 2b–d), whereas other anions such as AcO^- , F^- , and Cl^- did not have such a capability (Fig. S2). It should be noted that the gel triggered by SO_4^{2-} only has a moderate stability, while those triggered by other oxoanions remained stable for more than 3 months. Typical gelation conditions are given in Table S1. A stable gel state could still exist even at a low molar ratio of ATP or NO_3^- to M down to 1:1 at a total concentration of 100 mM. Optimal gelation pH in the presence of NO_3^- , SO_4^{2-} , and ATP was found to be 2–5 too. These observations suggest that a prerequisite for the gelation is that M should be protonated which would substantially enhance its hydrogen bonding with these oxoanions that may be strengthened by the electrostatic interactions too. Furthermore, pH and temperature were found to be reversible stimuli that trigger gel-to-sol and sol-to-gel state transitions. The high gelation selectivity towards these oxoanions is understood in terms of their at least 3 oxygen atoms as hydrogen bonding acceptors (Fig. 1a) versus those anions without oxygen atom or AcO^- anion with only

2 oxygen atoms. FESEM images of the hydrogels triggered by ATP and SO_4^{2-} show sheet-like micro-/nanoscale structures which are different from those triggered by NO_3^- and PO_4^{3-} (Fig. 2). It was observed that the gelation triggered by these oxoanions was independent of the solution ionic strength maintained by NaCl over 0 to 1.0 M (Fig. S3), indicating that the gelation is indeed facilitated by electrostatic and hydrogen bonding interactions that operate in a synergistic manner. It deserves pointing out that such a gelation triggered by oxoanions is unusual, since in most reported anion-tuning gel systems anions play a role of weakening or preventing gelation, normally leading to a gel-to-sol state transition.^{8a,c,10} To the best of our knowledge, very few examples involving anion induced or enhanced gelation have so far been reported.¹¹

X-ray diffraction (XRD) studies of the $\text{M}(\text{H}^+)_m\text{-XO}_y^{n-}$ xerogels show their crystalline character (Fig. S4), despite being anion dependent, indicating that different crystal natures exist in these xerogels. Attempts at single crystal structure analysis have been not successful presumably due to their nano-/microscale sizes. The characteristic infrared (IR) spectral peaks of oxoanions appearing in corresponding washed xerogels are indicative that anions bind with protonated M (Fig. S5–S8),¹² which is further confirmed by energy dispersive X-ray (EDX) spectroscopy featured by specific elements such as P, N, and S (Fig. S9–S12). These facts, together with the elemental analysis data (Table S2) that show binding ratios of M to $\text{NO}_3^-/\text{PO}_4^{3-}$, M to ATP, and M to SO_4^{2-} close to 1:1, 2:1, and 3:2, respectively, enable us to tentatively assume the formation of $[\text{M}(\text{H}^+)_m\text{-XO}_y^{n-}]$ supramolecular polymeric chains, suggesting that the oxoanions indeed served as “bridges” for the self-assembly (Fig. 1a).

Interestingly, similar nano-/microscale structures were observed at lower total concentration of M and XO_y^{n-} when gelation did not occur. For example, FESEM and TEM results of $\text{M}(\text{H}^+)_m\text{-NO}_3^-$ sol with a molar ratio of 1:1 at a total concentration of 50 mM demonstrate that nano-/microscale fibers exist and are similar to those of the hydrogel counterpart (Fig. S13). It thus appears that self-assembling occurs even in the sol state, which may open up a new approach for selective sensing of melamine¹³ and/or oxoanions based on such $\text{M}(\text{H}^+)_m\text{-XO}_y^{n-}$ interactions.

In conclusion, we showed that melamine in acidic aqueous solutions can be triggered to self-assemble into superstructures and to gelate a large amount of water molecules by oxoanions such as PO_4^{3-} , NO_3^- , SO_4^{2-} , and ATP. Reportedly the biological toxicity of melamine likely results from the formation of M–CA or M–UA aggregates. Our findings are therefore expected to offer new clues to the understanding of the melamine related diseases in that some of the oxoanions that are present within the body especially in some dosing cases may be involved in the toxicity of melamine. Although many investigations have been reported on the melamine-based superstructures, melamine in these cases is in its neutral form. Our attempt to explore the supramolecular chemistry of protonated melamine in aqueous solutions may initiate a detailed survey of the capacity of melamine in its protonated forms. Furthermore, the observation of the $\text{M}(\text{H}^+)_m\text{-XO}_y^{n-}$ assembly may open up a promising approach for developing novel receptors selective for melamine and/or oxoanions in

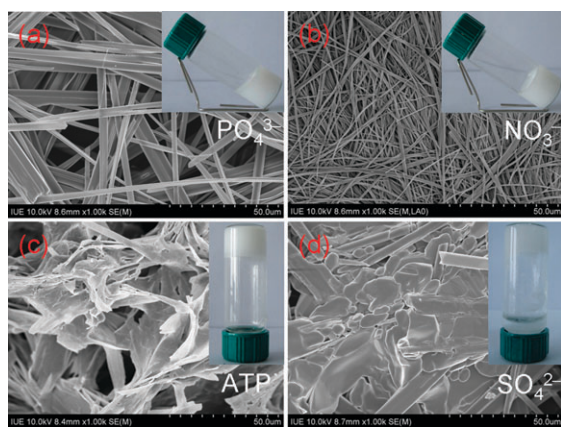


Fig. 2 FESEM images of xerogels from original hydrogels of protonated melamine triggered by PO_4^{3-} (a), NO_3^- (b), ATP (c), and SO_4^{2-} (d), respectively. Insets are corresponding photographs of the supramolecular hydrogels. Scale bar for (a–d) is 50 μm . Detailed gelation conditions: mixing 98.5 mg Na_3PO_4 and 12.6 mg melamine (a), 25.5 mg NaNO_3 and 6.3 mg melamine (b), 60.5 mg ATP and 12.6 mg melamine (c), and 63.9 mg Na_2SO_4 and 9.5 mg melamine (d) in 1 mL H_2O . In all cases pH is adjusted to 2–5 with dilute HCl or NaOH solution.

aqueous solutions and offer opportunities for fabricating electronically, optically, and biologically active new materials as well.

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