Formation of pH-Responsive Supramolecular Hydrogels in Basic Buffers: Self-assembly of Amphiphilic Tris-Urea

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Received June 30, 2021; accepted August 9, 2021

An amphiphilic tris-urea compound (I) containing hydrophilic resorcinol units was designed and synthesized. Compound 1 formed supramolecular hydrogels in basic buffers, such as glycine–NaOH, phosphate–NaOH, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–NaOH, and borate–NaOH. The optimum pH range of the buffer solution for gelation was 10–11 and insoluble suspensions or solutions were formed when the pH outside this range. When the borate–NaOH buffer was used, supramolecular hydrogels were formed over a wide pH range (7.5–11.0). The thermal stabilities and viscoelastic properties of the supramolecular hydrogels were determined from the gel-to-sol phase transition temperatures and rheological properties, respectively. The supramolecular hydrogel formed from compound 1 and the borate–NaOH buffer exhibited a pH-responsive reversible gel-to-sol phase transition property. Gel-to-sol phase transition could be achieved by adding NaOH and regulation of the sol was realized by adding an appropriate amount of boric acid. Increasing the amount of the acid resulted in a gel-to-sol phase transition.

Key words gel; self-assembly; phase transition; supramolecular chemistry; urea

Introduction

Hydrogels are flexible materials composed of chemically or physically intertwined polymer networks that entrap water. They are biocompatible because their major constituent is water. Hydrogels find their applications in a wide range of fields, such as agriculture, cosmetics, biological research, regenerative medicine, and pharmaceutical science. In the field of pharmaceutical science, hydrogels have been used as substrates for studying drug delivery systems (DDSs). Lioderm® is a hydrogel-based transdermal therapeutic system (TTS) composed of 5% lidocaine, which was developed as a therapeutic drug for post-herpetic neuralgia. Hydrogels are also used to support oral medication. For example, patients with dysphagia, who find it difficult to swallow tablets, have benefited from the use of hydrogels.

Supramolecular hydrogels are formed when small molecules, namely low molecular weight hydrogelators (LMWHGs), self-assemble in water, exploiting non-covalent interactions. The rational design of LMWHGs can help in the preparation of supramolecular hydrogels that show gel-to-sol and/or sol-to-gel phase transitions in response to specific external stimuli. For instance, supramolecular hydrogels that are pH-responsive have been developed by introducing pH-responsive functional groups in LMWHGs. pH-Responsive supramolecular hydrogels can be potentially used as substrates for studying DDSs as the internal pH of the body varies from site to site. Some typical examples are the pH of the stomach being acidic, that of the small intestine is almost neutral, and the tumor tissue is more acidic than normal tissue.

Progress has been made in the field of designing and synthesizing LMWHGs. It is now possible to design LMWHGs with the desired properties. LMWHGs with the desired properties. LMWHGs with the desired properties.

Results and Discussion

Compound 1 containing resorcinol moieties as the hydrophilic groups was synthesized (Chart 1). A condensation reaction between the isocyanate derived from 3,5-bis(tert-butylimethylsilyloxy)aniline (2) and 1,3,5-tris(3-aminophenoxymethyl)benzene (3) derived from 1,3,5-tris(bromomethyl)benzene produced the C3-symmetric tris-urea (4) in 76% yield. Subsequent acidic desilylation of 4 produced the desired compound 1 in 94% yield. Desilylation of 4 under general reaction conditions in the presence of fluoride ions resulted in the formation of trace amount of unidentified side-product. Compound 1 was characterized using 1H-NMR spectroscopy, 13C-NMR spectroscopy, and electrospray ionization mass spectrometry (ESI-MS).

The gelation abilities of 1 was determined using alkaline buffers, such as glycine–NaOH, phosphate–NaOH,
4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)–NaOH, and borate–NaOH. Mixtures of 1 and buffer solutions were thermally dissolved and cooled to ambient temperatures. Gel formation was studied by conducting the inverted tube test and a mixture adhered to the top of the inverted glass vial was defined as a gel (G). The results obtained from the gelation experiments and the minimum gelation concentration (MGC) of 1 are presented in Table 1.

A supramolecular hydrogel was not formed when 1 was mixed with pure water and acid buffers, whereas it was formed with the glycine–NaOH buffer (pH = 10.0; MGC = 0.3 wt%). However, an insoluble suspension was obtained when the glycine–NaOH buffer at pH 8.5 was used, indicating that the gelation ability depends on the pH of the system. A similar trend was observed with the phosphate–NaOH and HEPES–NaOH buffers. With the phosphate–NaOH buffer, an insoluble suspension was obtained at pH 9.2, a supramolecular hydrogel was formed at pH 10.0 (MGC = 0.5 wt%), and a solution was obtained at pH 12.0. With the HEPES–NaOH buffer, an insoluble suspension was obtained at pH 9.5, a supramolecular hydrogel was formed in the pH range 10.0–11.0 (MGC = 0.3 wt% at pH 10.0, MGC = 0.1 wt% at pH 10.6, and MGC = 0.2 wt% at pH 11.0), and a solution was obtained at pH 12.0. Mixtures of 1 and the borate–NaOH buffers yielded supramolecular hydrogels in the pH range 7.5–11.0. The MGCs of all the supramolecular hydrogels were ≤1.0 wt%. Transparent gels were formed in the pH range 8.5–10.0 (MGC = 0.1 wt%).

The thermal stabilities of the supramolecular hydrogels formed from the tris-urea 1 in HEPES–NaOH buffer and borate–NaOH buffer were determined from the gel-to-sol phase transition temperature (T<sub>gel</sub>) using the inverse flow method (Table 1). With the HEPES–NaOH buffer, the T<sub>gel</sub> values of the prepared supramolecular hydrogels at each MGC were 61 °C at pH 10.0, 92 °C at pH 10.6, and 107 °C at pH 11.0. The thermal stabilities of the supramolecular hydrogels increased with an increase in the basicity of the system. 32) The T<sub>gel</sub> values of the 0.5 wt% supramolecular hydrogels were >110 °C in the pH range 10.0–11.0, which increased with the increasing concentration of 1. This is a typical characteristic of supramolecular hydrogels. With the borate–NaOH buffer, the T<sub>gel</sub> values of the prepared supramolecular hydrogels at each MGC were 99 °C at pH 8.5, 108 °C at pH 9.0, 68 °C at pH 9.5, and 66 °C at pH 10.0. The thermal stabilities of the supramolecular hydrogels decreased as the basicity of the system increased. Further, the T<sub>gel</sub> values of the 0.5 wt% supramolecular hydrogels were >110 °C in the pH range 8.5–9.0, 93 °C at pH 9.5, and 85 °C at
that the pH was outside that range. We concluded that buffers, supramolecular hydrogels were obtained at pH 10.0–11.0, and insoluble suspensions or solutions were obtained when the pH was in the range 7.0–9.5, most of the phenolic hydroxy groups present in I were in their protonated (neutral) states. Under these conditions, compound I was not sufficiently hydrophilic to form gels. When the pH was approximately 10.0, only one of the hydroxy groups in the resorcinol moieties present in I is deprotonated. Under these conditions, the attractive phenol-phenoxide interactions present between the nanofibers may favor the process of gelation. At pH ≥12.0, both the hydroxy groups in resorcinol moieties present in I were deprotonated. Thus, the electrostatic repulsive forces generated by the phenoxide ions could potentially hinder the attractive interactions between the nanofibers.

Our results show that a mixture of I and the borate–NaOH buffer could be used to produce supramolecular hydrogels over a wide pH range. This could be attributed to the formation of “covalent” B–O bonds (borate ester) and/or “non-covalent” intermolecular hydrogen bonds. IR spectrum of a xerogel prepared from the freeze-drying supramolecular hydrogel of I and borate–NaOH buffer was measured. The peaks corresponding to the B–O stretch (at approximately 1350 cm\(^{-1}\)), C–O stretch (1220–1240 cm\(^{-1}\)), and out-of-plane vibration (640–680 cm\(^{-1}\)) are known as characteristic peaks of borate esters. However, they could not be clearly observed in the IR spectrum of the xerogel formed from I. Therefore, it was assumed that quantitative amounts of borate esters were not formed.

For glycine–NaOH, phosphate–NaOH, and HEPES–NaOH buffers, supramolecular hydrogels were obtained at pH 10.0–11.0, and insoluble suspensions or solutions were obtained when the pH was outside that range. We concluded that the \(pK_a\) values of the resorcinol moieties (\(pK_{a1} = 9.30; pK_{a2} = 11.06\)) determine the optimal pH required for gelation. When the pH was in the range 7.0–9.5, most of the phenolic hydroxy groups present in I were in their protonated (neutral) states. Under these conditions, compound I was not sufficiently hydrophilic to form gels. When the pH was approximately 10.0, only one of the hydroxy groups in the resorcinol moieties present in I is deprotonated. Under these conditions, the attractive phenol-phenoxide interactions present between the nanofibers may favor the process of gelation. At pH ≥12.0, both the hydroxy groups in resorcinol moieties present in I were deprotonated. Thus, the electrostatic repulsive forces generated by the phenoxide ions could potentially hinder the attractive interactions between the nanofibers.

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### Table 1. Gelation Properties of the Tris-Urea I

| Buffer          | pH   | Gelation test\(b\) (MGC) | \(T_{gel}\) \(c\) (concentration of I) |
|-----------------|------|--------------------------|-------------------------------------|
| H\(_2\)O        | around 7 | P                         |                                     |
| Glycine–NaOH    | 8.5  | G (0.3 wt%)               |                                     |
| Phosphate–NaOH  | 10.0 | G (0.5 wt%)               |                                     |
|                 | 12.0 | S                        |                                     |
| HEPES–NaOH      | 9.5  | P                        |                                     |
|                 | 10.0 | G (0.3 wt%)               | 61 °C (0.3 wt%)                     |
|                 | 10.6 | G (0.1 wt%)               | >110 °C (0.5 wt%)                   |
|                 | 11.0 | G (0.2 wt%)               | >110 °C (0.3 wt%)                   |
|                 | 12.0 | S                        |                                     |
| Borate–NaOH     | 7.0  | P                        |                                     |
|                 | 7.5  | G (0.25 wt%)              |                                     |
|                 | 8.5  | G (0.1 wt%)               | 99 °C (0.1 wt%)                     |
|                 | 9.0  | G (0.1 wt%)               | >110 °C (0.3 wt%)                   |
|                 | 9.5  | G (0.1 wt%)               | 108 °C (0.1 wt%)                    |
|                 | 10.0 | G (0.1 wt%)               | >110 °C (0.5 wt%)                   |
|                 | 11.0 | G (1.0 wt%)               | 85 °C (0.5 wt%)                     |
|                 | 12.0 | S                        |                                     |

\(a\) Each experiment was performed in buffers of concentration 50 mM. \(b\) Results obtained from the gelation tests and the minimum gelation concentration (MGC). \(c\) Gel-to-sol phase transition temperature \(T_{gel}\) of the supramolecular hydrogel formed from I.
3). The $T_{gel}$ values of the supramolecular hydrogels formed from a mixture of 1 and the borate–NaOH buffer at pH 8.5 and pH 9.0 were higher than those at pH 9.5 and 10.0 (Table 1); the former two were more thermally stable. This was because the phenolic hydroxy groups of the resorcinol moieties were in their protonated (neutral) states at pH values of 8.5 and 9.0. The formation of stable hydrogels could be attributed to the attractive interactions between the phenolic hydroxy groups and the borate ions. At pH values of 9.5 and 10.0, the phenolic hydroxy groups were partially deprotonated; thus, the thermal stabilities of the supramolecular hydrogels were compromised. The poor thermal stability can be attributed to the electrostatic repulsion between the phenoxide ions and the borate ions.

The pH-responsive gel-to-sol phase transition of the supramolecular hydrogel prepared from a mixture of 1, borate–NaOH buffer (pH = 8.5), and alizarin yellow R was observed, where alizarin yellow R was used as a pH indicator (Fig. 4). An aqueous solution of alizarin yellow R appears yellow at pH ≤ 10 and reddish-purple at pH ≥ 12. The color of the prepared supramolecular hydrogel was yellow at pH 8.5 (Fig. 4a). Adding NaOH to the supramolecular hydrogel resulted in a reddish-purple solution (pH = 12) (Fig. 4b). Further, when boric acid was added to the solution, an orange gel (pH = 10) was obtained (Fig. 4c), and increasing the amount of boric acid resulted in a yellow suspension (pH < 7) (Fig. 4d).

**Conclusion**

We developed a supramolecular hydrogel from a tris-urea 1 bearing resorcinol units as the hydrophilic groups. Mixtures of compound 1 and basic buffer solutions formed supramolecular hydrogels in the pH range 10–11. When borate–NaOH buffer was used, supramolecular hydrogels were formed over a wide pH range (7.5–11.0). The supramolecular hydrogels formed from a mixture of 1 and basic buffers were highly thermostable and exhibited reversible pH-dependent gel-to-sol phase transition. The development of supramolecular hydrogels responsive to various stimuli and their applications prospects are under investigation in our laboratory.

**Experimental**

Chemicals and solvents required were obtained from commercial suppliers. $^{1}$H- and $^{13}$C-NMR spectra were recorded on a JEOL JNM-ECZ-500 spectrometer. Mass spectra were measured on a JEOL JSM-6510LV spectrometer. SEM studies were carried out on a JEOL JNM-ECZ-500 spectrometer. Rheology measurements were performed by a TA Instruments DHR 2.

**Synthesis of Tris-Urea (1)** To a solution of 3,5-bis((tert-butyl)dimethylsilyloxy)aniline (2, 99.4 mg, 0.281 mmol) in 1,2-dichloroethane (0.55 mL) was added triphosgene (85.2 mg, 0.287 mmol) in 1,2-dichloroethane (0.55 mL) and Et$_3$N (90.0 µL, 0.645 mmol), successively under argon atmosphere at 0°C. The reaction mixture was stirred at room temperature for 15 min. The solvent was removed under reduced pressure and the crude product was obtained as white solid.

A solution of 3,5-bis((3-aminophenoxymethyl)benzene (3, 41.7 mg, 94.4 µmol) in 1,2-dichloroethane (1.10 mL) was added to the isocyanate under argon atmosphere at 0°C. The reaction mixture was kept at 90°C for 15 h and then cooled to room temperature and neutralized with saturated aqueous NH$_4$Cl solution. The organic layer was washed with brine, and dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure, and the crude product was purified by column chromatography (SiO$_2$, hexane/ethyl acetate 1/2). The desired product 4 was obtained as a white amorphous powder (114.0 mg, 76%), mp 138.5–139.0°C; $^{1}$H-NMR (Acetone-$d_6$, 500 MHz) δ: 0.22 (36H, s), 0.98 (54H, s), 5.15 (6H, s), 6.03 (3H, t, $J = 2.1$ Hz), 6.68 (3H, dd, $J = 8.0$, 2.1 Hz), 6.80 (6H, d, $J = 2.1$ Hz), 7.08 (3H, br-d, $J = 8.0$ Hz), 7.17 (3H, t, $J = 8.0$ Hz), 7.33 (3H, t, $J = 2.1$ Hz), 7.56 (3H, s), 8.05 (3H, s), 8.08 (3H, s); $^{13}$C-NMR (Acetone-$d_6$, 125 MHz) δ: −4.2, 18.8, 26.1, 70.2, 104.7, 106.6, 106.8, 109.2, 112.2, 127.0, 130.4, 139.0, 142.0, 142.4, 153.1, 157.6, 160.3; high resolution (HR)MS-ESI (m/z) [M + Na]$^+$ Calc for C$_9$H$_{28}$N$_3$NaO$_{28}$S$_6$: 1601.7942. Found 1601.7942.

**Synthesis of Tris-Urea (1)** To a solution of 4 (567 mg, 0.356 mmol) in N,N-dimethylformamide (DMF) (21.0 mL)
was added 1×HCl (4.5 mL) under argon atmosphere at 0°C. Then the mixture was stirred at room temperature for 5 d and neutralized with saturated aqueous NaHCO₃ solution. The solvent was removed under reduced pressure, and the mixture was diluted with water and the precipitate was filtered off. The crude product was thoroughly washed with water, hexane and a small amount of ethyl acetate to give the desired product I as a off-white solid (305 mg, 94%), mp 151.5–152.0°C; 1H-NMR (Acetone-d₆, 500 MHz) δ: 5.13 (6H, s), 5.99 (3H, t, J = 2.1 Hz), 6.59 (6H, d, J = 2.1 Hz), 6.64 (4H, br-d, J = 8.1 Hz), 6.99 (3H, br-d, J = 8.1 Hz), 7.13 (3H, t, J = 8.1 Hz), 7.35 (3H, t, J = 2.2 Hz), 7.54 (3H, s), 7.89 (3H, s), 8.00 (3H, s), 8.10 (6H, s); 13C-NMR (Acetone-d₆, 125 MHz) δ: 70.2, 97.8, 98.3, 106.3, 109.3, 112.0, 126.9, 130.4, 139.0, 142.2, 142.4, 153.1, 159.6, 160.3; HRMS-ESI (m/z) [M + Na]+ Calcd for C₄₈H₄₂N₆Na₂O₁₂ 917.2758. Found 917.2766.

Gelation Experiment A mixture of 1 and buffer in a glass vial bottle was heated on a hot plate (150 °C) until dissolved. Obtained solution was gradually cooled to ambient temperature. Gel formation was evaluated by the inverted tube test. A mixture remaining at the top of an inverted glass vial bottle was defined as a gel.

Acknowledgments This work was supported by Grant-in-aid for the Scientific Research (No. 20K06977 for M. Yo; 17H06374 and 21K064852 for M. Ya) the Japan Society for the Promotion of Science (JSPS) or the Ministry of Education, Culture, Sports, Science and Technology (MEXT) and the NOVARTIS Foundation (Japan) for the Promotion of Science (for S.K.)

Conflict of Interest The authors declare no conflict of interest.

References and Notes
1) Guilherme M. R., Aouada F. A., Fajardo A. R., Martins A. F., Paulino A. T., Davi M. F. T., Rubira A. F., Muniz E. C., Eur. Polym. J., 72, 365–385 (2015).
2) Mitura S., Sionkowska A., Jaiswal A., J. Mater. Sci. Mater. Med., 31, 50 (2020).
3) Malda J., Visser J., Melchels F. P., Jüngst T., Hennink W. E., Dhert W. J. A., Groll J., Hutmacher D. W., Adv. Mater., 25, 5011–5028 (2013).
4) Annabi N., Tamayol A., Uquillas J. A., Akbari M., Bertassoni L. E., Cha C., Camci-Unal G., Dokmeci M. R., Peppas N. A., Adv. Mater., 26, 85–124 (2014).
5) Ahmed E. M., J. Adv. Res., 6, 105–121 (2015).
6) Peppas N. A., Bures P., Leobandung W., Ichikawa H., Eur. J. Pharm. Biopharm., 50, 27–46 (2000).
7) Hoare T. R., Kohane D. S., Polymer, 49, 1993–2007 (2008).
8) Gammaitoni A. R., Alvarez N. A., Galer B. S., J. Clin. Pharmacol., 43, 111–117 (2003).
9) Tomita T., Yamaguchi A., Nishimura N., Goto H., Sumiya K., Ichikawa H., Yamanaka M., Tsuchiyagaito J., Chem. Commun., 2005, 3615–3631 (2005).
10) Du X., Zhou J., Shi J., Xu B., Chem. Rev., 115, 13165–13170 (2015).
11) Hirst A. R., Escudier B., Miravitlles J. F., Smith D. K., Angew. Chem. Int. Ed., 47, 8002–8018 (2008).
12) Frisch H., Besenius P., Macromol. Rapid Commun., 36, 346–363 (2015).
13) Howie J., Sangaj N., Varghese S., Macromol. Biosci., 19, 800259 (2019).
14) Datta S., Bhattacharya S., Chem. Soc. Rev., 44, 5596–5637 (2015).
15) Peters G. M., Davi J. I., Chem. Soc. Rev., 45, 3188–3206 (2016).
16) Singh N., Kumar M., Miravitlles J. F., Uljin R. V., Escudier B., Chem. Eur. J., 23, 981–993 (2017).
17) Hanabusa K., Kawakami A., Kimura M., Shirai H., Chem. Lett., 26, 191–192 (1997).
18) van Gorp J. J., Vekeman J. A., Meijer E. W., J. Am. Chem. Soc., 124, 14759–14769 (2002).
19) Heeres A., van der Pol C., Stuart M., Frierger A., Ito M., van Esch J., J. Am. Chem. Soc., 125, 14252–14253 (2003).
20) van Bommel K. J., van der Pol C., Muizbeldt I., Frierger A., Heeres A., Meetsma A., Frierger B. L., van Esch J., Angew. Chem. Int. Ed., 43, 1663–1667 (2004).
21) Haino T., Tanaka M., Fukazawa Y., Chem. Commun., 2008, 468–470 (2008).
22) Yokota M., Kimura S., Yamanaka M., Chem. Eur. J., 27, 5601–5614 (2021).
23) Yamanaka M., Chem. Rec., 16, 768–782 (2016).
24) Yamanaka M., Yanai K., Zama Y., Tsuichiyagaito J., Yoshida M., Ishii A., Hasegawa M., Chem. Asian J., 10, 1299–1303 (2015).
25) Sakano T., Ohashi T., Yamanaka M., Kobayashi K., Org. Biomol. Chem., 13, 8359–8364 (2015).
26) Yamanaka M., Nakagawa T., Aoyama R., Nakamura T., Tetrahedron, 64, 11558–11567 (2008).
27) The Tgel values of the supramolecular hydrogels formed from 1 in HEPES–NaOH buffer: Tgel = 98 °C (pH = 10.6, 0.2 wt%); Tgel = 107 °C (pH = 11.0, 0.2 wt%); Tgel >110 °C (pH = 10.6, 0.3 wt%).
28) Wright A. J., Marangoni A. G., J. Am. Oil Chem. Soc., 83, 497–503 (2006).
29) Rambo B. M., Lavigne J. J., Chem. Mater., 19, 3732–3739 (2007).
30) Smith M. K., Northrop B. H., Chem. Mater., 26, 3781–3785 (2014).
31) Matsushima Y., Nishiyabu R., Takahashi N., Haruta M., Kimura H., Kubo Y., J. Mater. Chem. B., 22, 24124–24131 (2012).