Modifying a Known Gelator Scaffold for Nitrite Detection

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Modifying a Known Gelator Scaffold for Nitrite Detection

Danielle M. Zurcher, Yash J. Adhia, Julián Díaz Romero and Anne J. McNeil*

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The process of selecting and modifying a known gelator scaffold to develop a new nitrite-based sensor is described. Five new azo-sulfonate gelators were discovered and characterized. The most promising scaffold exhibits a stable diazonium intermediate, proceeds in a high yield, and gels nitrite-spiked tap, river, and pond water.

Small molecule-based gels are being explored for a variety of different applications. Identifying an appropriate gelator for a specific application can be quite simple if the final gel state is all that matters. For example, different growth factors have been added to known peptide-based gelators and used as scaffolds in tissue engineering. On the other hand, identifying an appropriate gelator can be quite challenging if the solution-to-gel phase transition is important to the application (e.g., sensing). In this case, one needs not only a gelator, but also a non-gelling, structurally related precursor. As a consequence, in our previous work, we found it easier to design a new gelator than to modify a known gelator scaffold to develop a light-responsive gelator can be quite challenging if the solution-to-gel phase transition is important to the application (e.g., sensing). In this case, one needs not only a gelator, but also a non-gelling, structurally related precursor. As a consequence, in our previous work, we found it easier to design a new gelator than to modify a known gelator scaffold to develop a new nitrite-based sensor. Five new azo-sulfonate gelators were discovered and characterized. The most promising scaffold exhibits a stable diazonium intermediate, proceeds in a high yield, and gels nitrite-spiked tap, river, and pond water.

Nitrite (NO$_2$) contamination of water is a global concern because of its detrimental effects on human health. Nitrite sensors based on electrochemical and colorimetric methods, among others, have been developed, however, sample pretreatment is often required. In contrast, sensors based on molecular gelation are portable and can operate in complex media (e.g., opaque samples). To develop a molecular gel-based sensor for nitrite, the first step involved identifying a chemical transformation that can be mediated by nitrite. Herein, the Griess reaction was chosen because of its high selectivity for nitrite, short reaction time, and high yield. In the original Griess reaction, sulfanilic acid reacts with nitrous acid to form a diazonium ion, which is then reacted with α-naphthylamine to generate a red-violet azo dye (eq 1).

The next step was to identify a gelator that can be formed via the Griess reaction. A search of the small molecule gel literature revealed a surprisingly large number of azo-containing organogelators and a few hydrogels. In most cases, an azobenzene moiety was added to a known gelator scaffold to generate a light-responsive transition (via the trans-to-cis azobenzene isomerization). Because the non-azo-functionalized precursor is also a gelator, most of these scaffolds are unsuitable for sensing. We focused our attention solely on hydrogels with scaffolds wherein the azo-functional group was part of the core structure (rather than an appendage). Only one promising scaffold fit these parameters: azo-sulfonates. These azo-compounds can be accessed via the Griess reaction between an aniline derivative and sodium 6-hydroxynaphthalene-2-sulfonate, which are both non-gelling in aqueous solutions (eq 2). Azosulfonates were reported to gel aqueous buffers, albeit at high concentrations. Thus, we began our studies by modifying this scaffold to develop a gelation-based nitrite sensor for environmental samples.

We focused our efforts on meta-substituted derivatives because Hamada and co-workers previously reported that most para-substituted derivatives were nongelators under aqueous conditions. In total, seven azosulfonates (3a-3g) were synthesized and screened for gelation (Table 1 and ESI). All seven compounds formed gels in either borax buffer (65 mM, pH = 13) or EtOH/buffer (9/1 v/v). Because the ultimate goal is to use aqueous samples from the environment, we focused on lowering the critical gel concentration (cgc) in neat borax buffer. The unsubstituted derivative (3g) was too soluble, so a single methyl- (3a), chloro- (3b) or trifluoromethyl- (3c) substituent was
introduced to increase the hydrophobicity.34 The most
hydrophobic compound (3c) within this series gave the lowest
cgc in borax buffer. Introducing a second, identical substituent
(3d–f) had a large effect on cgc when R = Cl and a surprisingly
small effect on cgc when R = CF3. Overall, the dichloro-
substituted derivative (3e) exhibited the lowest cgc, although 3b,
3e and 3f were only slightly higher.

Table 1. Critical gel concentrations (cgc) in different conditions

<table>
<thead>
<tr>
<th>Azosulfonate</th>
<th>EtOH/borax buffer (9/1, v/v)</th>
<th>Borax buffer</th>
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<tr>
<td>3a</td>
<td>23.5 ± 0.4</td>
<td>precipitate</td>
</tr>
<tr>
<td>3b</td>
<td>43 ± 3</td>
<td>29.4 ± 0.9'</td>
</tr>
<tr>
<td>3c</td>
<td>precipitate</td>
<td>24.2 ± 0.8</td>
</tr>
<tr>
<td>3d</td>
<td>35.5 ± 0.2</td>
<td>precipitate</td>
</tr>
<tr>
<td>3e</td>
<td>16.7 ± 0.6</td>
<td>21.3 ± 0.5</td>
</tr>
<tr>
<td>3f</td>
<td>precipitate</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>3g</td>
<td>30.0 ± 0.5</td>
<td>soluble</td>
</tr>
</tbody>
</table>

* The error reported as a standard deviation is based on 3 samples. Each
compound was screened for gelation at concentrations ≤ 2 wt%. The term
“precipitate” was used when any amount of precipitate was observed. 

The mechanical strength and morphology of gels of 3a–g were
characterized using rheology and scanning electron microscopy,
respectively.35 Rheological testing of all gels at 1.5 times the
cgc revealed the expected 10-fold (or larger) storage modulus
(G') relative to the loss modulus (G'"") in both the frequency and
oscillating stress sweep experiments, confirming its gel-like
nature (c.f., Figure 1A and ESI).36 Optical and scanning electron
microscopy was performed on each gel near or above its cgc to
determine the morphology.37 Consistent with most molecular
gels, anisotropic fibers of varying widths were observed (c.f.,
Figure 1B and ESI).

The Griess reaction is typically carried out at lower
temperatures (e.g., 0 °C), presumably due to concerns about
diazonium ion stability.14 Nevertheless, a practical and portable
sensor should operate at ambient temperatures. Thus, UV-vis
spectroscopy was used to monitor the rate of diazonium ion
formation (2a–g) and decomposition at room temperature.
Gratifyingly, diazonium ions 2b (Cl), 2e (CF3), 2f (Cl/CF3) and 2f
(CF3/CF3) were stable at ambient temperatures for at least an hour
(c.f., Figure 2A and ESI).1 H NMR spectroscopy was used to confirm
that a single, stable species was formed during the
reaction (Figure 2B). In contrast, the CH3-substituted derivatives
(2a, 2d) showed presumed loss of nitrogen after 2 min and the
unsubstituted derivative (2g) decomposed after 30 min. Based on
these results, our further studies focused solely on those gelators
formed through stable diazonium intermediates (i.e., 3b, 3c, 3e
and 3f).

One concern that emerged from the syntheses of 3a–g was the
moderate-to-low isolated yields (e.g., 17% yield for 3e, see ESI).
The sensitivity of a gel-based sensor depends on both the critical
gel concentration and the yield of the reaction used to generate
the gelator. For example, although 3e exhibited the lowest overall
cgc, its low reaction yield could ultimately make it less suitable in
the sensor platform. Because the low synthetic yields were
presumably due to the extensive purification required to remove
the excess salts, we used 1 H NMR spectroscopy to quantify the in
situ reaction yield for 3b, 3c, 3e, and 3f using an internal standard
(c.f., Figure 3 and ESI). Gratifyingly, all four reactions proceeded
with yields exceeding 85% (ESI).

Gelator 3e was ultimately selected for the sensor platform on
the basis of its low cgc, stable diazonium intermediate, and
high reaction conversion. We tested the nitrite sensor in different
water sources by spiking each sample with NaNO2 because the
natural [NO2−] in non-polluted water is low. Vials containing 1e

Figure 1. (A) Oscillating stress sweep for a gel of 3e (32 mM in borax
buffer, G' (•) and G'"") (·). (B) Scanning electron microscope image of a
gel of 3e (37 mM in EtOH/buffer (9/1, v/v)).

Figure 2. (A) Plot of the normalized absorbance versus wavelength for
the reaction of 1e (4.8 x 10^-4 mmol) with NaNO2 (4.8 x 10^-3 mmol) at
room temperature in 4 M aq. H2SO4 (0 min (black solid), 2 min (red
dashed) 10 min (green dash dot dot), 30 min (blue dot), 60 min (purple
dash dot)). (B) 1H NMR spectra (in d6-DMSO) acquired before (bottom)
and 15 min after (top) adding NaNO2 (0.03 mmol) to 1e (0.03 mmol) in 4
M aq. H2SO4 at rt.

Figure 3. 1H NMR spectra of the crude reaction mixture (without
workup) for the reaction of 1e (0.0078 mmol) with NaNO2 (0.0071 mmol)
and sodium 6-hydroxypropyl-2-sulfonate (0.0078 mmol) in
deuterated borax buffer (top), and the corresponding starting materials
and products under the same solvent conditions.
(suspended in 4 M H$_2$SO$_4$) were treated with the spiked water samples for 10 min, followed by adding sodium 6-hydroxynaphthalene-2-sulfonate (in borax buffer). The resulting samples were heated to dissolve all solids and then allowed to cool to room temperature. The bright red/orange color is indicative of azosulfonate formation (Figure 4).

As evident in Figure 4, the gel-based nitrite sensor proved to be quite robust as it gelled tap water, river and pond water, as well as water drawn from a muddy pond. The non-spiked water samples serve as a negative control. Surprisingly, the cge determined under these reaction conditions was significantly lower (9.3 mM) than that observed with the isolated (and purified) compound (21.3 mM). We suspected that the change in pH from our screening conditions (pH = 13) to the reaction conditions (pH = 9) might play a role given the acidic hydrazine proton ($K_a$ ~ 11). Eventually, a similar cge was observed for isolated 3e when the pH matched those of the reaction conditions (9.5 ± 0.3 mM, see ESI). Overall, the detection limit in these studies was 500 ppm, which is above the EPA minimum set for safe drinking water (1 ppm).

We have previously demonstrated that using a smaller vial leads to a lower cge, which can be attributed to an increase in the surface area between the container and the gel. Herein, the detection limit dropped to 90 ppm using a 1.5 mL vial (instead of 4 mL, see ESI). Further reducing this detection limit will require either identifying a better gelator or a reaction that is catalytic in nitrite, wherein each nitrite produces more than one gelator molecule.

**Figure 4.** Gel formation is observed after a heat/cool cycle when tap water, river water, pond water, and muddy pond water containing NaN$_2$ were reacted with 1e for 10 min, followed by sodium 6-hydroxynaphthalene-2-sulfonate (see ESI for experimental details).

In conclusion, these studies reveal that known gelators can be successfully modified and then utilized in targeted applications. Herein we demonstrated that by altering the substituents on the precursor aniline ring, we were able to develop a nitrite sensor that is operable under ambient temperatures in aqueous, environmental samples. The impact of these studies is expected to be large considering that over a thousand small molecules have been reported to form gels and each of these compounds represent potential starting points for developing other gelation-based applications.

**Notes and references**

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† Electronic Supplementary Information (ESI) available: synthetic procedures, spectroscopic data, gel screening data, rheological data, microscopy data, thermogravimetric analysis data, UV-vis spectroscopic data, and in situ gelation procedures. See DOI: 10.1039/b000000x/


(20) Ureas: (a) S. van der Laan, B. L. Feringa, R. M. Kellogg and J. van Esch, Langmuir, 2002, 18, 7136–7140; (b) M. de Loos, J. van Esch, certified standard solution from SPEX CertiPrep Group (ESI).


(25) The strength and stability of these gels were unaffected by adding excess sodium nitrite (ESI).

(26) Gels of 3c were transparent within the limits of our optical microscope. In addition, the scanning electron microscope images only revealed salt crystals from the buffer solution.


(28) The detection limit is based on the total volume of the gel sample and not the volume of nitrite-contaminated water.

(29) We screened for gelation in borax buffer because the Griess reaction was previously reported to proceed in high yield in this solvent system. For reference, see: E. Kalatzis, J. Chem. Soc., 1967, 273–277.


(31) The detection limit is based on the total volume of the gel sample and not the volume of nitrite-contaminated water.