

Multiwell plates loaded with fluorescent hydrogel sensors for measuring pH and glucose concentration

Boaz Vilozny, Alexander Schiller,^{a*} Ritchie A. Wessling, and Bakthan Singaram*

Department of Chemistry and Biochemistry, University of California, 1156 High Street, Santa Cruz, CA 95064 (USA)

^a *Friedrich-Schiller-University Jena, Institute for Inorganic and Analytical Chemistry, August-Bebel Str 2, 07743 Jena (Germany)*

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Materials. The initiator, 2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride (ABIP), was purchased from Wako Pure Chemical Industries (VA-044, MW 323.33). *N,N*-dimethylacrylamide (DMAA) inhibited with 400 ppm MeHQ (methoxyhydroquinone) was purchased from Aldrich. *N,N'*-methylenebisacrylamide (MBA), Ultrapure, was purchased from Polysciences. Sodium metabisulfite, Na₂S₂O₅, (97%) was purchased from Sigma. Potassium persulfate, K₂S₂O₈, (99%) and iron(II) sulfate heptahydrate, FeSO₄·7H₂O, (99%) were purchased from Aldrich. Sulfuric acid, ACS reagent grade, was purchased from Acros. Phosphate buffered saline (PBS) contained 10 mM phosphate and 140 mM sodium chloride, and the pH was adjusted with HCl or NaOH (22 °C). Experiments were carried out at room temperature (22 °C). Stock DMAA/MBA solutions were prepared in advance and stored at 4 °C. All other solutions were freshly prepared. Water that had been purified by a Milli-Q[®] 18-MΩ-cm system was used to make all solutions. All dilutions were carried out using volumetric flasks or micropipettes with a precision of > 0.1%. Stock solutions were diluted as required immediately before use.

Polymerization at ambient temperature with ABIP initiator. To a new 20 mL vial were added a stir bar, DMAA (0.498 g), MBA (0.010 g), 1 M HCl (0.050 mL), and water to bring the total volume to 5.00 mL. The ABIP initiator (50 mg) was added, the solution mixed rapidly for 10 seconds, then cooled in an ice bath while sparging 5 min with argon. The solution was then capped tightly and monitored for gelation while stirring at either room temperature (22±2 °C) or in a water bath 37±1 °C.

Gelation trials to evaluate initiator concentrations. To a new 20 mL scintillation vial with a small stir bar were added DMAA (0.5 mL containing 2% w/v MBA), sulfuric acid (0.05 mL, 0.25 N), and an aliquot of sodium metabisulfite (500 mM). Water was then added so that the final volume, after addition of the remaining initiators, would be 5.00 mL. The solution was

allowed to stir 1 min, then an aliquot of potassium persulfate (50 mM) was added and the stirring solution was capped. If after ten minutes no gelation occurred, an aliquot of iron(II) sulfate (10 or 50 μL , 18 mM) was added and the stirring solution capped again. The gelation point was considered to be the time, after iron addition, at which the stir bar became immobilized in the matrix. Gels to be analyzed were set aside for 2 h to complete polymerization. Amounts of monomer, crosslinker, and acid were kept constant for all trials. The molar ratio of metabisulfite to persulfate in this series of experiments was 1:1.

Evaluation of gel formation (percent polymer). After 2 h curing time, a portion of 10 mL acetone was added to the vial and the gel was broken up with a spatula. The stir bar was removed, and the contents of the vial were transferred to a vacuum oven-dried, pre-tared, fluted filter funnel. Two more portions of 5 mL acetone were used to wash the vial and transfer the remaining material to ensure quantitative recovery. After gravity filtration and air-drying 3 h, the filter paper with the gel was placed in a vacuum oven at 40 °C for 16 h. Percent polymer formed was calculated using the formula

$$\% \text{ conversion} = W_{\text{dry}}/W_{\text{theor}} \times 100$$

where W_{dry} is the weight of the dried gel, and W_{theor} is the combined weight of the matrix monomer and crosslinker.

Preparation of Gel Disks for Swelling Measurements. To a new 4 mL screw-cap vial with a micro-stir bar were added DMAA (96 mg), MBA (2 to 8 mg), water (0.900 mL), sulfuric acid (0.01 mL, 0.25 N), metabisulfite (0.03 mL, 100 mM), persulfate (0.01 mL, 50 mM), and ferrous iron (0.03 mL, 1.0 mM). The vial was then capped and stirred 5 min. Persulfate was then added and the mixture was drawn up into a 3 mL plastic syringe and cured 2 h at room temperature (22 °C). The syringe was then frozen in dry ice/acetone, the tip cut off, and the gel

extruded and sliced into disks of approximate thickness 1.3 mm. The diameter of several disks was measured with a ruler. The disks were then placed in water for 18 h and the diameter measured again. Swelling was characterized by percent diameter increase:

$$\% \text{ Increase} = \frac{D_{sw} - D_i}{D_i} \times 100\%$$

where D_i and D_{sw} are the diameters of the disks before and after swelling, respectively.

Preparation of thin film gels. Thin films were formed between two glass plates treated with dichlorodimethylsilane, separated by a 0.005 inch spacer. Polymerization with ABIP was done in an air-tight cassette as described previously. **Error! Bookmark not defined.** The redox-initiated gels were cast without any precautions against air. After curing, the plates were separated and the film removed. It was then cut into pieces, which were soaked in water for 24 h to remove unreacted monomer and other impurities.

Thin-film hydrogel with radical initiator. To a 2 mL vial were added DMAA/MBA solution (0.388 mL, 20 mg/mL MBA in DMAA), MABP (23.0 mg, 0.02 mmols), water (0.602 mL), sulfuric acid (0.01 mL, 0.25 N), APTS-BUMA (1.3 mg, 0.002 mmols). ABIP (2.4 mg) was then added to this solution, following which it was deoxygenated with three cycles of freezing, thawing under vacuum, and purging with argon. The monomer solution was then injected into an argon-purged, air-tight mold, which was then placed in two sealed plastic bags and immersed in a water bath at 37 °C for 24 h.

Gel Recipe Optimization. In the case of both the highest and lowest concentrations of initiator used, the monomer solutions did not form a gel. Instead, the solution became more viscous. For the high initiator concentrations, (10 and 25 mM) we interpret this to be the result of rapid chain initiation and termination preventing crosslinking, thereby producing highly

branched soluble polymers. All of the other trials resulted in clear, homogeneous gels with gelation occurring in a matter of minutes. The successful gel trials were evaluated after 2 h curing time to measure the extent of polymerization by dry polymer weight. Only the gel with the lowest concentration of initiators and catalyst (trial 7) gave incomplete polymerization, indicated by liquid monomer solution remaining at the surface of the gel. The remaining gels were found to have between 94% and quantitative polymer yields based on dry weight after washing to remove unreacted monomer.

Gels were prepared by pre-mixing the co-monomer solution, persulfate, and metabisulfite in a capped vial, and gelation was triggered by adding ferrous sulfate. It was noticed that the gelation time seemed to depend on the amount of time the solution was mixed before adding the iron catalyst. We hypothesized that this resulted from the reaction of metabisulfite with oxygen in the air. These solutions were initiated by adding metabisulfite at varying concentrations from 0.5 mM to 3.3 mM. Because each mole of metabisulfite is hydrolyzed to give two moles bisulfite at low pH, the reducing agent concentrations effectively ranged from 1 mM to 7 mM. Increased metabisulfite concentrations resulted in the shortest gelation times, indicating the bisulfite is indeed consumed by oxygen in the flask during the reaction. In the 1 mL headspace in the capped flask for these reactions, there were approximately 3 mmols oxygen present. A trial with an equivalent amount of bisulfite (3 mmols total) required 50 min to form a gel, while the trial with 20 mmols total bisulfite gelled in 1.5 min. A gel formulation with excess metabisulfite (3.5 mmols metabisulfite, 0.5 mmols persulfate, 0.03 mmols iron(II)sulfate) was further evaluated for reproducibility of gelation and polymer yield. For three replicates of this recipe, the gel time was between 1.2 and 1.5 minutes. The percent polymerization (measured by dry polymer weight) was

98±3% within a 90% confidence interval. We chose this initiator protocol to study further incorporation of sensing components in redox-initiated hydrogels.

Effect of polymerization on fluorophore. The choice of using excess metabisulfite in the redox polymerization reaction proved essential for formulations including the dye monomer. Mixing a pyrene dye monomer (APTS-BUMA) with the hydrogel monomers without initiators resulted in a green solution that was highly fluorescent. Using a non-optimized redox system (2.5 mM each of metabisulfite and persulfate, 0.04 mM Fe²⁺) rapidly produced a gel that turned from green to orange over several minutes and was non-fluorescent, indicating degradation or quenching of the dye. Lowering the initiator concentrations five-fold gave the same result. Only the polymerization with excess metabisulfite (Table 2) produced a green fluorescent gel, showing the need for gelation in a reducing environment.

Table S1. Gelation times and polymer yield for initial hydrogel formulations. Gels contained 10% monomer DMAA (w/w) and 2% crosslinker MBA. Monomer solutions were adjusted to pH 3 and stirred with metabisulfite and persulfate before triggering the reaction with iron(II)sulfate.

Trial	[initiator] (mM)			Gel Time (min) ^a	% polymer ^b
	Metabisulfite	Persulfate	Fe ^{II}		
1	25	25	0.2	No gel	ND
2	10	10	0.2	No gel	ND
3	5	5	0.2	0.5	100
4	2.5	2.5	0.2	0.5	98
5	0.5	0.5	0.2	0.5	94
6	2.5	2.5	0.04	1	99
7	0.5	0.5	0.04	2	ND
8	0	0.5	0.2	No gel	ND

a) gel time measured from addition of Fe^{II}

b) % polymer determined by dry weight after 2 h curing time. ND = not determined.

Table S2. Effect of initiator ratios on fluorescence of APTS-BUMA. Hydrogels contained 10% DMAA with 2% crosslinker (monomer weight), and gelation was triggered by adding iron(II)sulfate.

Trial	[metabisulfite/persulfate/Fe ^{II}] (mM)	Color	Notes
D1	0 / 0 / 0	Green	Fluorescent
D2	2.5 / 2.5 / 0.04	Orange	non-fluorescent
D3	0.5 / 0.5 / 0.03	Orange	non-fluorescent
D4	3.3 / 0.5 / 0.03	Green	Fluorescent

We evaluated swelling in hydrogels that differed only in the amount of the crosslinker added. Rods of gels containing only DMAA and MBA (10% monomer weight) were prepared and sliced into disks. The diameter was measured immediately after polymerization and again after several days in water. As seen in Figure 5, there was a near-linear correlation between % diameter increase and amount of crosslinker over the range from 1 to 5 mole % MBA.

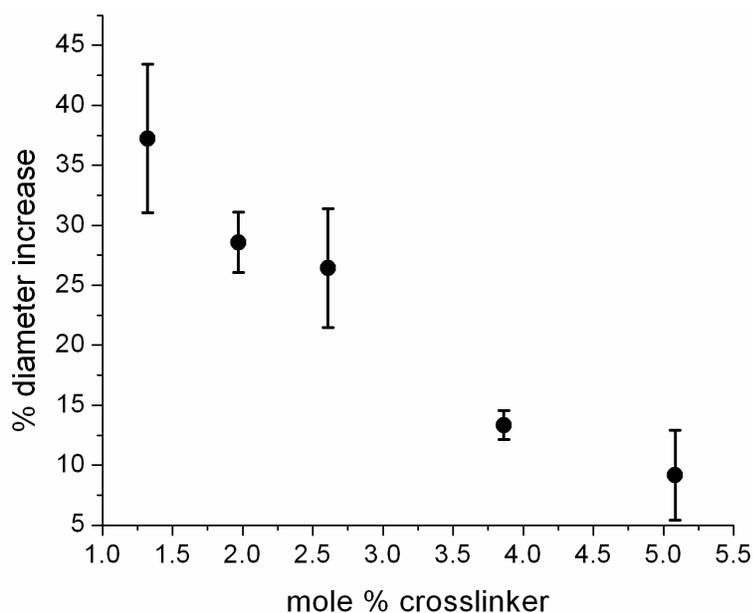


Figure S1. Swelling of hydrogels as a function of crosslinking. The diameter increase was used to measure swelling in hydrogel disks made with 10% DMAA and varying amounts MBA crosslinker. The error bars reflect the standard deviation among several disks (N=4).

Because some swelling is desired in order to keep the hydrogels seated in the micro-wells, we chose a level of 4 mole % (6% monomer weight) MBA in the recipe for further evaluation.

One more modification to our gel formulation protocol was necessary in adapting it from thin-films to multi-well plates in order to control the rate of polymerization. We found that the start of polymerization could be controlled in a reproducible fashion by cooling the monomer mixture in an ice bath, adding all the initiators, then pipetting the cold solutions into a multi-well plate. Using this technique, auto-polymerization of the monomer mixture was not a problem and the solutions gelled as they warmed to ambient temperature. It was found that the polymerization could be made faster by enclosing the plate in an argon-purged bag (1 hour gel time), but even in air the system yielded high conversion in 3 to 4 h.

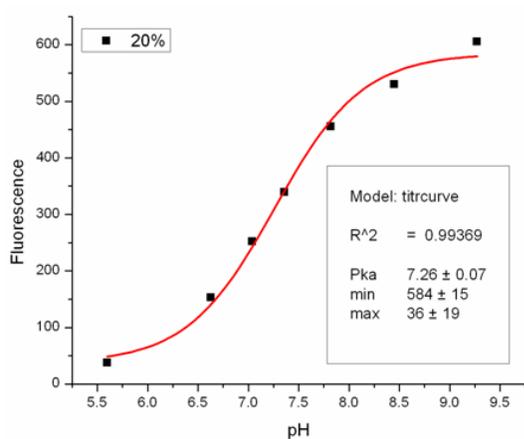


Figure S2. Curve fitting with a pH-sensitive hydrogel array to find apparent pK_a . See experimental section for details. Apparent $pK_a = 7.26$, $R^2 = 0.99369$.

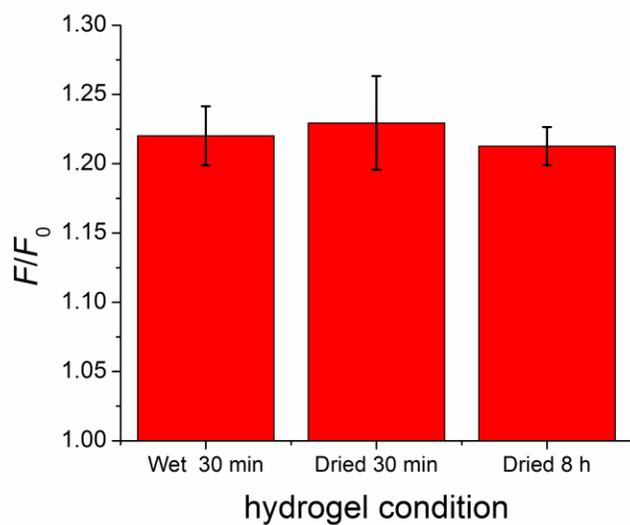


Figure S3. Signal modulation for wet and rehydrated hydrogels. The gels were exposed to solutions of 10 mM glucose in PBS for either 30 minutes (left and center) or 8 h (right). Error bars reflect the standard deviation of triplicate wells. P7 BV4-141