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Article

## Poly(*N*-isopropylacrylamide) Hydrogels for Storage and Delivery of Reagents to Paper-Based Analytical Devices

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## **Supplementary Materials**

## **Purification of PNIPAM via Thermocycling**

Thermocycle trends for all three diameters of PNIPAM are shown in Figure S1. Thermocycling the PNIPAM is paramount for achieving a hydrogel that delivers consistent masses of fluid. The variations in the mass percent delivered within the first four thermocycles were due most likely to expulsion of the sol from the hydrogel. The leveling off of the mass percent delivered after four cycles suggests the completion of sol expulsion. To assess whether the sol had been removed from the gel, samples of hydrogel of each diameter (5.35 mm, 7.24 mm, and 11.03 mm) were weighed before and after the heating step. The mass of the samples averaged 0.13 g, 0.15 g, and 0.35 g for diameters of 5.35 mm, 7.24 mm, and 11.03 mm, respectively. Large standard deviations were observed in the mass percent delivered for all three diameters, which can be attributed to the relatively large variation in the mass of the samples comes from the difficulty of cutting reproducible sample masses by hand. When small sample sizes are cut for assessment, differences in the masses of these small sample sizes caused large relative differences in the mass of fluid expelled when the thermally excited chains collapsed. This was a problem we faced when working with PNIPAM throughout this project. Ultimately, thermocycling is necessary for quality control, but is difficult to assess quantitatively.



**Figure S1.** Plot of the mass percent expelled versus the number of thermocycles for all three diameters of PNIPAM. Data points represent the mean of 3 measurements and error bars represent one standard deviation from the mean.

## Fluid Delivery from PNIPAM

Samples of PNIPAM of three diameters, (5.35 mm, 7.24 mm, and 11.03 mm) were used to study the fluid delivery characteristics of the hydrogel at temperatures above the LCST as a function of the sample's mass and time. For assessing the fluid delivery characteristics as a function of mass, PNIPAM samples of each diameter were prepared by dissecting portions of the hydrogel using a razor blade. Samples were produced in mass ranges of 0.0229 to 1.9178 g, 0.0776 to 3.0743 g and 0.2241 to 8.2954 g, for the diameters of 5.35 mm, 7.24 mm and 11.03 mm respectively. Each sample was weighed and then placed on a digital hot plate (Thermo Scientific MAREC) at 40 °C for 12 min. The sample was weighed again after the heating step. The difference of the initial and final mass of the PNIPAM hydrogel was used to determine the mass of fluid delivered (Figure S2).

To study the effect of the duration of the heat exposure on the amount of fluid delivered by PNIPAM, two samples for diameters 5.35 mm (with initial masses of 0.767 g and 1.75 g), 7.24 mm (with initial masses of 1.25 g and 3.49 g), and 11.03 mm diameter gel (with initial masses 1.56 g and 6.12 g) were prepared. All prepared samples were reused throughout the series of time trials. Samples were weighed prior to the time trial, then heated at 40 °C on a digital hot plate for a specified time ranging from 2 to 60 min, and weighed after exposure. Then, the samples were cooled and rehydrated in a room temperature DI H<sub>2</sub>O bath for 1 h before being reused (Figure S3).



**Figure S2.** Plot of the initial mass of a PNIPAM sample versus the average mass of fluid expelled. The mass of fluid delivered increases with an increase in the mass of the sample. Diminishing returns are observed. Data points represent the mean of three measurements. Data was fit with logarithmic trend lines to aid the eye.



**Figure S3.** Plot of heating time versus average mass delivered from two samples of each diameter. Each data point represents a single measurement. Data was fit with logarithmic trend lines to aid the eye. The diameter and mass of each sample is provided in the graph.

**Table S1.** Fluorescence intensity values for the delivery of antibody and DNA to microPADs. Values represent the mean of three measurements and the uncertainty is reported as one standard deviation from the mean.

	Fluorescence intensity	
C <sub>antibody</sub> / mg●L <sup>-1</sup>	PNIPAM	Solution
93.8	$2324 \pm 1026$	$2118 \pm 867$
750	$24188 \pm 2975$	$24208 \pm 1361$
[DNA] / µM		
0.994	$8344 \pm 3000$	$7131 \pm 1000$
7.95	$27357 \pm 300$	$27187 \pm 2000$