



Cite this: *Nanoscale*, 2015, 7, 2784

## Poly (*N*-isopropylacrylamide)-*co*-(acrylic acid) microgel/Ag nanoparticle hybrids for the colorimetric sensing of H<sub>2</sub>O<sub>2</sub>†

De-Man Han,<sup>a,b</sup> Qiang Matthew Zhang<sup>a</sup> and Michael J. Serpe<sup>\*a</sup>

Poly (*N*-isopropylacrylamide)-*co*-(acrylic acid) (pNIPAm-*co*-AAc) microgels composed of Ag nanoparticles (Ag NPs) have been synthesized and employed for the colorimetric sensing of H<sub>2</sub>O<sub>2</sub>. Each pNIPAm-*co*-AAc microgel, which exhibited a diameter of ~800 nm, contained multiple Ag NPs (diameter of ~5 nm), and solutions of these hybrid materials showed a UV-vis absorption band at ~400 nm. This is due to the excitation of the Ag NP surface plasmon. We go on to show that the intensity of this absorption band is dependent on the concentration of H<sub>2</sub>O<sub>2</sub> in solution. Specifically, in the presence of H<sub>2</sub>O<sub>2</sub> the magnitude of the absorption peak dramatically decreases in a linear fashion over the concentration range of 0.30 to 3.00 μM H<sub>2</sub>O<sub>2</sub> ( $r^2 = 0.9918$ ). We go on to show that the response is selective for H<sub>2</sub>O<sub>2</sub> and can still function in complex mixtures, *e.g.*, we showed that the response is still robust in milk samples. While Ag NPs themselves can exhibit similar responses, this system has many benefits including sample processing and long term stability – *i.e.*, Ag NPs are destabilized in solutions of a certain pH, and aggregate readily. Our microgel/Ag NP hybrids have been shown to be extremely stable and are easily purified prior to use by simple centrifugation/washing protocols. This system is simple and straightforward to use, is low cost, and can be used in complex media, which makes it practical for analyzing complex biological and environmental samples.

Received 15th October 2014,  
Accepted 27th December 2014

DOI: 10.1039/c4nr06093h

www.rsc.org/nanoscale

## Introduction

H<sub>2</sub>O<sub>2</sub> is one of the most important intermediate species involved in many environmental and biological processes, and is ubiquitous in various industrial processes and medicine.<sup>1–3</sup> Because of this, the detection and quantitation of H<sub>2</sub>O<sub>2</sub> in samples is of extreme importance. Multiple detection methods and schemes such as fluorimetry,<sup>4</sup> spectrophotometry,<sup>5</sup> chemiluminescence<sup>6</sup> and titrimetry<sup>7</sup> have been employed in the past for the detection of H<sub>2</sub>O<sub>2</sub>. Among them, many biosensors have been developed on the basis of electrocatalysis of immobilized enzymes as a result of H<sub>2</sub>O<sub>2</sub> reduction.<sup>8</sup> However, the enzyme-based H<sub>2</sub>O<sub>2</sub> sensors have some disadvantages. For example, the enzymes are expensive, the activity is dependent on environmental conditions, and they suffer from a short shelf life.<sup>9</sup> With the development of nanotechnology, nanomaterials for sensing H<sub>2</sub>O<sub>2</sub> have been receiving increased attention with

many advantage including enhanced catalytic activities and electron transfer, and stability. Among metals, silver nanoparticles (Ag NPs) display excellent electrocatalytic activity towards the reduction of H<sub>2</sub>O<sub>2</sub>.<sup>10–12</sup> It has also been shown that Ag NPs are useful at enhancing electron transfer for the reduction of H<sub>2</sub>O<sub>2</sub>, believed to be a result of larger paths for charge transport channels.<sup>13</sup> Furthermore, Ag NPs have many features such as highly sensitivity, biocompatibility and cost effectiveness when compared to other noble metal nanoparticles.<sup>14</sup>

While Ag NPs have some advantages, they suffer from instability in water, and tend to aggregate. This hinders their long-term storage and processibility, ultimately limiting their application. Therefore, it is important to protect the Ag NPs from aggregation if they are to have future utility. The aggregation of metal nanoparticles can be prevented either by electrostatic or steric stabilization,<sup>15–17</sup> and many methods have been developed to solve these problems by using stabilizing agents or some protecting carrier systems.<sup>18–21</sup> For example, Filippo *et al.*<sup>22</sup> and Guascito *et al.*<sup>23</sup> have used polyvinyl alcohol to stabilize Ag NPs and used for H<sub>2</sub>O<sub>2</sub> sensing. Zhao *et al.*<sup>8</sup> Wang *et al.*<sup>24</sup> and Sophia *et al.*<sup>25</sup> employed PVP (polyvinyl pyrrolidone) as protecting agent for Ag NPs to be used in H<sub>2</sub>O<sub>2</sub> sensing. Recently, poly (*N*-isopropylacrylamide) (pNIPAm)

<sup>a</sup>Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2. E-mail: michael.serpe@ualberta.ca, hdm@tzc.edu.cn;

Fax: +1 780 492 8231, +86 576 88660353; Tel: +1 780 492 5778, +86 576 88660353

<sup>b</sup>Department of Chemistry, Taizhou University, Taizhou, China, 318000

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c4nr06093h



copolymer microgels have been used successfully to stabilize Ag NPs. Dong *et al.*<sup>26</sup> used poly (*N*-isopropylacrylamide)-*co*-(acrylic acid) (pNIPAm-*co*-AAc) microgel particles with different crosslinking densities as microreactors for the *in situ* fabrication of Ag NPs. Khan *et al.*<sup>18</sup> fabricated Ag NPs in poly (*N*-isopropylacrylamide-*co*-methacrylic acid) microgels for rapid degradation of 4-nitrophenol in aqueous medium. Finally, Ballauff *et al.* synthesized poly(styrene) core pNIPAm shell particles, with the shell doped with Ag NPs and studied their ability to reduce 4-nitrophenol as a function of temperature.<sup>27</sup> While many have generated Ag NPs in pNIPAm-based microgels, there have been no reports on their use as a colorimetric sensor for H<sub>2</sub>O<sub>2</sub> in solution.

As established above, the determination of H<sub>2</sub>O<sub>2</sub> concentration in solutions is of importance for many reasons. There are many established methods for conducting such an analysis, such as fluorimetry (FL), chemiluminescence (CL) and electrochemical methods. While this is the case, these techniques involve the use of expensive equipment that must be operated by skilled operators. In this submission, we developed a pNIPAm-*co*-AAc/Ag NPs hybrid microgel-based system for conducting such measurements by the use of a simple UV-Vis spectrophotometer. Not only is the system easier to use, and the Ag NPs easier to process, compared to the others mentioned above, but the microgel-based systems were shown to be very sensitive to H<sub>2</sub>O<sub>2</sub> concentration in solution, even when using complex media, *e.g.*, milk samples.

## Materials and methods

### Chemicals

The monomer *N*-isopropylacrylamide (NIPAm) was purchased from TCI (Portland, OR) and purified by recrystallization from hexanes (ACS reagent grade, EMD, Gibbstown, NJ). *N,N'*-Methylenebisacrylamide (BIS) (99%), acrylic acid (AAc) (99%), ammonium persulfate (APS) (98%), silver nitrate, sodium borohydride and hydrogen peroxide were obtained from Sigma-Aldrich (Oakville, Ontario) and were used as received. All other chemicals were of analytical grade. Deionized (DI) water with a resistivity of 18.2 MΩ cm was obtained from a Milli-Q Plus system from Millipore (Billerica, MA), and filtered through a 0.2 μm filter prior to use.

### Synthesis of pNIPAm-*co*-AAc microgels

pNIPAm-*co*-AAc microgels were synthesized utilizing established procedures.<sup>28</sup> Specifically, the monomer mixture was comprised of 75% NIPAm and 20% AAc with 5% BIS as the crosslinker. The monomer, NIPAm (10.54 mmol), and the crosslinker, BIS (0.703 mmol), were dissolved in DI water (99 mL) with stirring in a small beaker. The mixture was filtered through a 0.2 μm filter affixed to a 20 mL syringe into a 250 mL, 3-necked round bottom flask. The flask was then fitted with a temperature probe, a condenser/N<sub>2</sub> outlet, stir bar, and a N<sub>2</sub> inlet. The monomer solution was purged with N<sub>2</sub> gas for about 1 h with stirring, while the temperature was

allowed to reach 70 °C. AAc (2.812 mmol) was added to the heated mixture with a micropipette. An aqueous solution of APS (0.046 g in 1.0 mL) was delivered to the monomer solution with a transfer pipette to initiate the reaction, and reaction was continued for 3 h. Following completion of the reaction, the reaction mixture was filtered through glass wool to remove any large aggregates. The coagulum was rinsed with deionized water and the reaction solution was diluted to about 100 mL. Aliquots of these particles (12 mL) in centrifuge tubes were centrifuged at a speed of 10 000 relative centrifugal force (rcf) at 20 °C to produce a pellet. The supernatant was removed from the pellet of particles, which were then resuspended to their original volume (12 mL) using deionized water. This process was completed a total of five times to remove the unreacted monomer and linear polymer from the microgels. Finally, pellet of particles in centrifuge tubes was collected and suspended to 30 mL by adding deionized water for the next synthesis step.

### Synthesis of pNIPAm-*co*-AAc/Ag NPs hybrid microgels

Hybrid microgels were synthesized by reduction of silver ions within the polymer network of pNIPAm-*co*-AAc microgels. Briefly, 5 mL of a previously prepared and purified microgels solution and 35 mL of deionized water were added into a three-necked round bottom flask (100 mL) equipped with N<sub>2</sub> inlet and condenser. The pH of this solution was adjusted to 8.62 by the addition of 0.01 M NaOH. Then 0.05 mL of an aqueous 0.1 M AgNO<sub>3</sub> solution was added and stirred for 30 minutes at 25 °C followed by the drop wise addition of 0.01 g of NaBH<sub>4</sub> dissolved in 5 mL of deionized water. The solution color immediately changed from colorless to yellow, and was further stirred for one hour. Then the resultant mixture was centrifuged at a speed of 10 000 relative centrifugal force (rcf) at 25 °C to produce a pellet composed of the pNIPAm-*co*-AAc/Ag NP hybrid microgels. The centrifuge process was completed a total of five times and the final centrifuged pellet was diluted to 10 mL with deionized water for analysis.

### Characterization

UV-Vis spectra of the as prepared Ag NP hybrid microgel solutions were collected using a 8452A Diode Array Spectrophotometer (Hewlett Packard, USA). Transmission electron microscopy (TEM) images were recorded using JSM-1200EX II TEM (JEOL, Ltd, Tokyo, Japan) system. The purified Ag NP hybrid microgels were deposited onto a TEM grid with a thin layer of carbon. Scanning electron microscopy (SEM) images were obtained with a JSM-6010LA JEOL (Peabody, MA) SEM. An energy-dispersive X-ray (EDX) (Philips, Roanoke, VA, U.S.A.) was used to confirm the compositions of the Ag NPs in the hybrid microgels.

### Sensor of H<sub>2</sub>O<sub>2</sub>

For the quantification of H<sub>2</sub>O<sub>2</sub> in solution, multiple reaction solutions were analyzed. Each reaction solution contained 200 μL of the pNIPAm-*co*-AAc/Ag NP hybrid microgels, and



various amounts of a diluted  $\text{H}_2\text{O}_2$  solution was added to result in a specific  $\text{H}_2\text{O}_2$  concentration. After mixing *via* gentle vortexing the solutions were allowed to remain for 1 minute. After 1 minute the solutions were all diluted to 3.00 mL with deionized water and added to a 1 cm path length quartz cuvette and the UV-vis spectrum collected from 200 nm to 700 nm. The spectra revealed a strong absorption at 400 nm, and the intensity at this wavelength was related to the solution  $\text{H}_2\text{O}_2$  concentration.

### Analysis of a milk sample

To verify the utility of this method for  $\text{H}_2\text{O}_2$  sensing in complex fluids, a local milk sample (from Edmonton, Alberta, Canada) was analyzed. Before analysis, the sample was prepared as follows: 5.00 mL of milk or 5.00 mL of milk spiked with a specific amount of  $\text{H}_2\text{O}_2$  was added with 20 mL of methanol (containing 0.1 M sodium acetate) respectively. After sonication for 10 min, each sample mixture was equilibrated for 30 min to allow the proteins to precipitate. Every solution was further centrifuged at 10 000 rcf for 10 min. After that, 20 mL of *n*-hexane was added to the each supernatant solution to extract fat and the aqueous portion was collected for analysis, respectively. Specifically, 200  $\mu\text{L}$  of the pNIPAm-*co*-AAc/Ag NP hybrid microgels and a certain volume of aqueous pre-treated sample were mixed and allowed to incubate for 1 min, the sample was then diluted to 3.00 mL with deionized water and analyzed *via* a UV-vis spectrometer.

## Results and discussion

pNIPAm-*co*-AAc microgels were used as microreactors for the *in situ* synthesis of highly dispersed and stable Ag NPs, using  $\text{AgNO}_3$  as a precursor and  $\text{NaBH}_4$  as a reducing agent. The Ag ion loading was done at pH 8.62, which was shown to be the optimal pH (see ESI†). As can be seen in the SEM image in Fig. 1(a), microgels could be synthesized, and exhibit a spherical shape. The Ag composition of the microgels was confirmed using EDX. As can be seen in Fig. 1(b), the EDX spectrum of the microgels (the exact location of spectrum collection is shown in Fig. 1(a)) showed very strong peaks, which are indicative of Ag. To further confirm that the microgels contained Ag NPs, TEM images were collected. As can be seen in Fig. 1(c, d), the microgel network contained Ag NPs with diameters of  $\sim 5$  nm. Another image of the Ag NPs in the microgels can be seen in ESI.† These data are evidence that the microgel network structure provides an ideal scaffold for the nucleation and growth of Ag nanoparticles.

We went on to investigate the optical properties of solutions of the pNIPAm-*co*-AAc/Ag NP hybrid microgel solutions. The UV-Vis spectra of the pNIPAm-*co*-AAc/Ag NP hybrid microgels and the pNIPAm-*co*-AAc microgels were collected, and as can be seen in Fig. 2, the solutions containing the pNIPAm-*co*-AAc/Ag NP hybrid microgels exhibited a strong absorption at  $\sim 400$  nm, while the other solution didn't exhibit an absorption in this region. The absorption at  $\sim 400$  nm is attributed to the

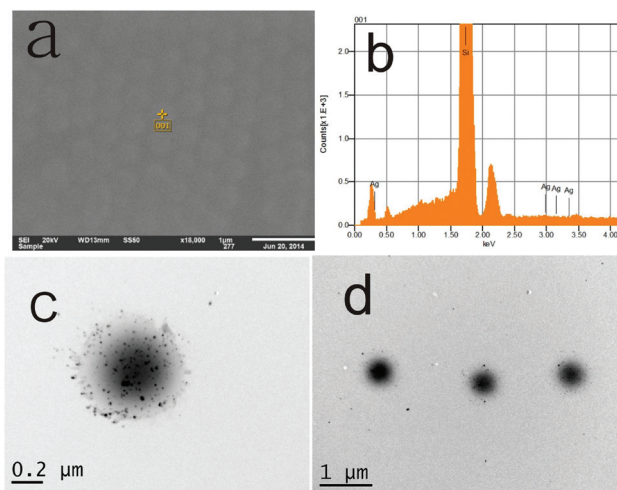


Fig. 1 (a) SEM, (b) EDX spectrum, and (c,d) TEM for the generated pNIPAm-*co*-AAc/Ag NP hybrid microgels.

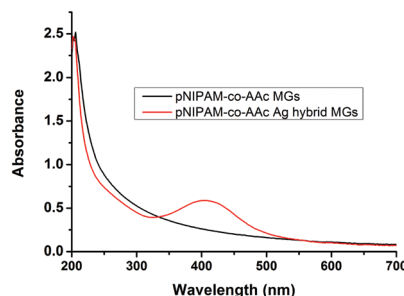


Fig. 2 UV-vis absorption spectra for (red) pNIPAm-*co*-AAc/Ag NP hybrid microgels and (black) pNIPAm-*co*-AAc microgels.

surface plasmon resonance of the Ag NPs. This has also been confirmed by Wu *et al.* who explained that the broad plasmon band near 400 nm refers to the surface plasmon absorbance band of Ag NPs immobilized in the microgels.<sup>29</sup> We would like to point out here that the Ag NPs in the microgels were extremely stable, compared to Ag NPs in solution without microgels for stabilization. Specifically, we have shown that the Ag NPs in the microgels were stable when stored in the dark at room temperature, while the Ag NPs synthesized in the same manner, but without the microgels, aggregated and settled out of solution within 5 minutes, see ESI.† Furthermore, Ag NPs in the microgels were also stable at the different temperature (Fig. 3).

After characterization of the pNIPAm-*co*-AAc/Ag NP hybrid microgels, we went on to prove their sensitivity to the presence of  $\text{H}_2\text{O}_2$ . As can be seen in Fig. 4, the color of the pNIPAm-*co*-AAc/Ag NP hybrid microgel solution changed from yellow to colorless upon the introduction of  $\text{H}_2\text{O}_2$ , making the solution concentration 1 mM  $\text{H}_2\text{O}_2$ . We note here that bubbles were generated due to the generation of oxygen as a result of the catalytic reaction between Ag and  $\text{H}_2\text{O}_2$ . The proposed reaction



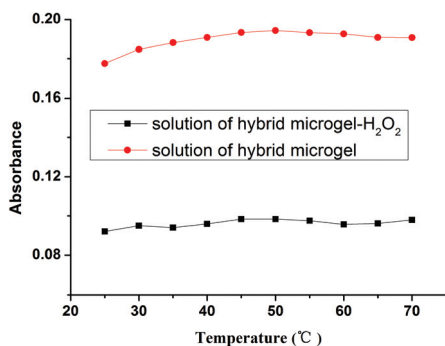


Fig. 3 The effect of temperature on the absorbance of (●, red) pNIPAm-co-AAC/Ag NP hybrid microgel solutions and (■, black) after addition of H<sub>2</sub>O<sub>2</sub> (total concentration 3.00 mM).

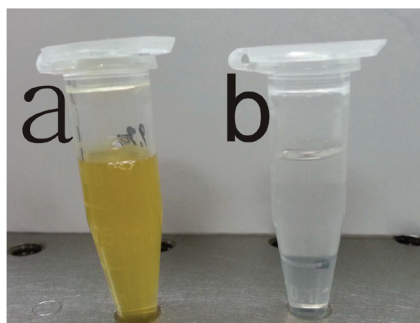
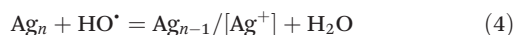


Fig. 4 Photographs of solutions containing pNIPAm-co-AAC/Ag NP hybrid microgels (a) before and (b) after the addition of H<sub>2</sub>O<sub>2</sub>.

mechanism is shown below, as presented by Jiang and coworkers:<sup>30</sup>



Since the pNIPAm-co-AAC/Ag NP hybrid microgels were shown to be sensitive to H<sub>2</sub>O<sub>2</sub>, we went further and characterized the system's response to various concentrations of H<sub>2</sub>O<sub>2</sub>. To accomplish this, the same amount of pNIPAm-co-AAC/Ag NP hybrid microgels was added to solutions of various concentrations of H<sub>2</sub>O<sub>2</sub> and the UV-vis spectrum collected. As can be seen in Fig. 5, the absorbance value at 400 nm decreases as the solution concentration of H<sub>2</sub>O<sub>2</sub> increased. Importantly, it is noted that the baseline for the curves is stable from sample to sample. From the UV-vis spectra, we were able to plot the variation in absorbance at 400 nm as a function of H<sub>2</sub>O<sub>2</sub>. From the data in Fig. 6, it can be seen that the response is extremely linear in the range of 0.3–3.0 μM H<sub>2</sub>O<sub>2</sub> ( $r^2 = 0.9918$ ). The effect of pH on the catalytic reaction has been studied (Fig. 7), and the results showed that the pH value did not affect the UV-vis absorbance significantly over a wide range. The absorbance of

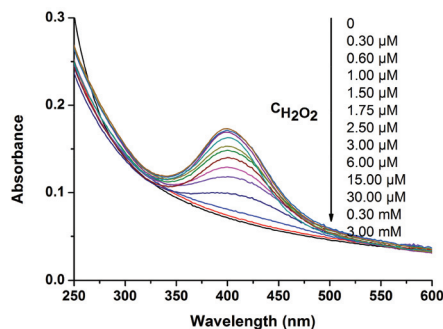


Fig. 5 UV-vis absorption spectra of solutions containing pNIPAm-co-AAC/Ag NP hybrid microgels before, and after the addition of the indicated amounts of H<sub>2</sub>O<sub>2</sub>.

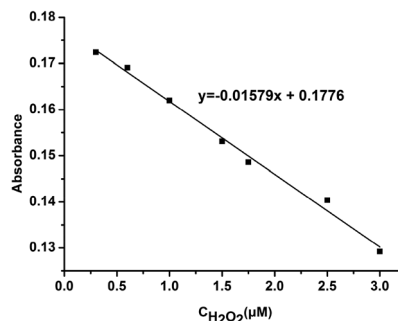


Fig. 6 The variation of the absorbance at 400 nm as a function H<sub>2</sub>O<sub>2</sub> concentration.

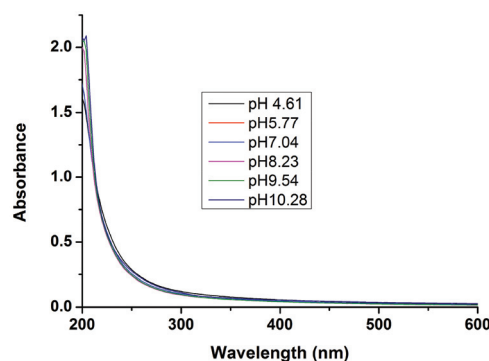
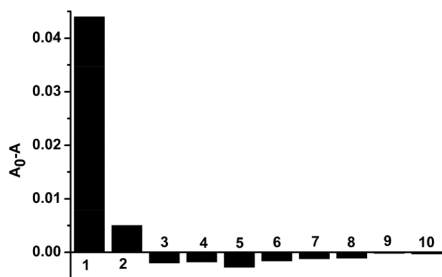


Fig. 7 The effect of pH value on the catalytic reaction between the pNIPAm-co-AAC/Ag NP hybrid microgel after addition of H<sub>2</sub>O<sub>2</sub> (total concentration 3.00 mM).

the catalytic reaction was also shown to be stable at different temperatures (Fig. 3).

Finally, we went on to evaluate the selectivity of the pNIPAm-co-AAC/Ag NP hybrid microgels to H<sub>2</sub>O<sub>2</sub>. To investigate the selectivity, we exposed pNIPAm-co-AAC/Ag NP hybrid microgels to individual solutions containing 50 μM of: glucose, ascorbic acid, uric acid, Cu<sup>2+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, Fe<sup>3+</sup>, Mg<sup>2+</sup>, and K<sup>+</sup>. Fig. 8 reveals that the absorbance values did not change considerably (by comparing the absorbance at 400 nm before and





**Fig. 8** The selectivity of the Ag NPs hybrid microgels toward H<sub>2</sub>O<sub>2</sub>. (1) H<sub>2</sub>O<sub>2</sub> (1 μM), (2) ascorbic acid (50 μM), (3) Glucose (50 μM), (4) uric acid (50 μM), (5) Ca<sup>2+</sup> (50 μM), (6) Fe<sup>3+</sup> (50 μM), (7) Cu<sup>2+</sup> (50 μM), (8) Mg<sup>2+</sup> (50 μM), (9) Na<sup>+</sup> (50 μM), (10) K<sup>+</sup> (50 μM).

**Table 1** Results of hydrogen peroxide assay in milk samples

Sample	Conc. spiked H <sub>2</sub> O <sub>2</sub> (μM)	Detected (μM, average value)	Average recovery (% , n = 3)	Standard deviation (% , n = 3)
Milk	0	Not detected	/	/
	0.88	0.81	92.0%	3.7%
	1.76	1.60	90.9%	4.4%

after exposure) in response to these interfering species, while it changed greatly in the presence of 1 μM H<sub>2</sub>O<sub>2</sub>.

Based on the above results, it is clear that the pNIPAm-co-AAc/Ag NP hybrid microgels can be used to quantify the amount of H<sub>2</sub>O<sub>2</sub> in aqueous solutions. Next, we wanted to show that the pNIPAm-co-AAc/Ag NP hybrid microgels could be used to quantify H<sub>2</sub>O<sub>2</sub> in complex media. To evaluate this, we have performed the above sensing strategy in a milk sample. The milk samples spiked with different concentrations of H<sub>2</sub>O<sub>2</sub> were treated as detailed in the Experimental section above to remove protein and fat from the samples, and the aqueous portion of the treated sample was collected for testing. The results from three separate test at each indicated concentration are shown in Table 1. As can be seen, H<sub>2</sub>O<sub>2</sub> could be detected in each sample that was spiked, and the recoveries (amount H<sub>2</sub>O<sub>2</sub> detected compared to the original amount of H<sub>2</sub>O<sub>2</sub> in the milk) were on average 90.9% and 92.0% with standard deviations below 4.5%, which confirms satisfactory accuracy and precision of the proposed method. This method may have great potential applications for hydrogen peroxide assay in food and biological samples.

## Conclusions

In conclusion, we have demonstrated that a simple colorimetric sensor for the determination of H<sub>2</sub>O<sub>2</sub> in aqueous samples, as well as complex media, could be generated. The system is made by generating Ag NPs inside the polymer network of pNIPAm-co-AAc microgels, which affords solutions with a yellow color and a strong UV-vis absorption at 400 nm.

We showed that the absorbance at 400 nm decreased in the presence of H<sub>2</sub>O<sub>2</sub>, leading to the solution color disappearing. This was due to the H<sub>2</sub>O<sub>2</sub>-induced autocatalytic oxidization of the Ag NPs. We also showed that the absorbance at 400 nm was linearly dependent of H<sub>2</sub>O<sub>2</sub> concentration over a wide range of concentrations, and was selective to H<sub>2</sub>O<sub>2</sub>. Finally, showed that the method could be used to detect H<sub>2</sub>O<sub>2</sub> in milk samples. This approach represents a fast and inexpensive method for H<sub>2</sub>O<sub>2</sub> detection, and has clear advantages over Ag NPs themselves including enhanced stability, long shelf life, and easy processibility. These systems may find use for analyzing medical samples, for environmental monitoring and for food quality control.

## Competing interest

The authors declare no competing financial interest.

## Acknowledgements

M.J.S. acknowledges funding from the University of Alberta (the Department of Chemistry and the Faculty of Science), the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation (CFI), the Alberta Advanced Education & Technology Small Equipment grants Program (AET/SEGP). D.H. acknowledges funding from the National Natural Science Foundation of China (no. 21375092), China Postdoctoral Science Foundation (no. 2013M530248), and State Key Laboratory of Analytical Chemistry for Life Science Funds (SKLACLS1207).

## Notes and references

- B. Wang, J. Zhang, Z. Pan, X. Tao and H. Wang, A Novel Hydrogen Peroxide Sensor Based on the Direct Electron Transfer of Horseradish Peroxidase Immobilized on Silica-hydroxyapatite Hybrid Film, *Biosens. Bioelectron.*, 2009, **24**, 1141–1145.
- M. C. Y. Chang, A. Pralle, E. Y. Isacoff and C. J. Chang, A Selective, Cell-permeable Optical Probe for Hydrogen Peroxide in Living Cells, *J. Am. Chem. Soc.*, 2004, **126**, 15392–15393.
- O. S. Wolfbeis, A. Durkop, M. Wu and Z. Lin, A Europium-ion-based Luminescent Sensing Probe for Hydrogen Peroxide, *Angew. Chem., Int. Ed.*, 2002, **41**, 4495–4498.
- I. Mori, K. Takasaki, Y. Fujita and T. Matsuo, Selective and Sensitive Fluorometric Determinations of Cobalt (II) and Hydrogen Peroxide with Fluorescein-hydrazide, *Talanta*, 1998, **47**, 631–637.
- M. Hoshino, S. Kamino, M. Doi, S. Takada, S. Mitani, R. Yanagihara, M. Asano, T. Yamaguchi and Y. Fujita, Spectrophotometric Determination of Hydrogen Peroxide with Osmium(VIII) and m-carboxyphenylfluorone, *Spectrochim. Acta Part A*, 2014, **117**, 814–816.



- 6 S. Hanaoka, J. Lin and M. Yamada, Chemiluminescent Flow Sensor for Hydrogen Peroxide Based on the Decomposition of Hydrogen Peroxide Catalyzed by Cobalt (II)-ethanolamine Complex Immobilized on Resin, *Anal. Chim. Acta*, 2001, **426**, 57–64.
- 7 E. C. Hurdis and H. Romeyn, Accuracy of Determination of Hydrogen Peroxide by Cerate Oxidimetry, *Anal. Chem.*, 1954, **26**, 320–325.
- 8 B. Zhao, Z. Liu, G. Liu, Z. Li, J. Wang and X. Dong, Silver Microspheres for Application as Hydrogen Peroxide Sensor, *Electrochem. Commun.*, 2009, **11**, 1707–1710.
- 9 G. Zou and H. Ju, Electrogenerated Chemiluminescence from a CdSe Nanocrystal Film and Its Sensing Application in Aqueous Solution, *Anal. Chem.*, 2004, **76**, 6871–6876.
- 10 J. Sophia and G. Muralidharan, Preparation of Vinyl Polymer Stabilized Silver Nanospheres for Electro-analytical Determination of H<sub>2</sub>O<sub>2</sub>, *Sens. Actuators, B*, 2014, **193**, 149–156.
- 11 K. Cui, Y. H. Song, Y. Yao, Z. Z. Huang and L. Wang, A Novel Hydrogen Peroxide Sensor Based on Ag Nanoparticles Electrodeposited on DNA-networks Modified Glassy Carbon Electrode, *Electrochem. Commun.*, 2008, **10**, 663–667.
- 12 A. Safavi, N. Maleki and E. Farjami, Electrodeposited Silver Nanoparticles on Carbon Ionic Liquid Electrode for Electrocatalytic Sensing of Hydrogen Peroxide, *Electroanalysis*, 2009, **21**, 1533–1538.
- 13 X. C. Song, X. Wang, Y. F. Zheng, R. Ma and H. Y. Yin, A Hydrogen Peroxide Electrochemical Sensor Based on Ag Nanoparticles Grown on ITO Substrate, *J. Nanopart. Res.*, 2011, **13**, 5449–5455.
- 14 L. Chen, X. Fu, W. Lu and L. Chen, Highly Sensitive and Selective Colorimetric Sensing of Hg<sup>2+</sup> Based on the Morphology Transition of Silver Nanoprisms, *ACS Appl. Mater. Interfaces*, 2013, **5**, 284–290.
- 15 S. Laurent, D. Forge, M. Port, A. Roch, C. Robic, L. V. Elst and R. N. Muller, Magnetic Iron Oxide Nanoparticles: Synthesis, Stabilization, Vectorization, Physicochemical Characterizations, and Biological Applications, *Chem. Rev.*, 2008, **108**(6), 2064–2110.
- 16 N. Mandy, E. Grulke and T. Druffel, Breakage of TiO<sub>2</sub> Agglomerates in Electrostatically Stabilized Aqueous Dispersions, *Powder Technol.*, 2005, **160**, 121–126.
- 17 M. Karg and T. Hellweg, Smart Inorganic/organic Hybrid Microgels: Synthesis and Characterisation, *J. Mater. Chem.*, 2009, **19**, 8714–8727.
- 18 S. R. Khan, Z. H. Farooqi, M. Ajmal, M. Siddiq and A. Khan, Synthesis, Characterization, and Silver Nanoparticles Fabrication in N-isopropylacrylamide-Based Polymer Microgels for Rapid Degradation of p-Nitrophenol, *J. Dispersion Sci. Technol.*, 2013, **34**, 1324–1333.
- 19 M. Karg and T. Hellweg, New “smart” Poly (NIPAM) Microgels and Nanoparticle Microgel Hybrids: Properties and Advances in Characterisation, *Curr. Opin. Colloid Interface Sci.*, 2009, **14**, 438–450.
- 20 M. Karg, Y. Lu, E. Carbo-Argibay, I. Pastoriza-Santos, J. Perez-Juste, L. M. Liz-Marzan and T. Hellweg, Multi-responsive Hybrid Colloids Based on Gold Nanorods and Poly (NIPAM-co-allylacetic acid) Microgels: Temperature and pH Tunable Plasmon Resonance, *Langmuir*, 2009, **25**, 3163–3167.
- 21 R. Contreras-Caceres, A. Sanchez-Iglesias, M. Karg, I. Pastoriza-Santos, J. Perez-Juste, J. Pacifico, T. Hellweg, A. Fernandez-Barbero and L. M. Liz-Marzan, Encapsulation and Growth of Gold Nanoparticles in Thermoresponsive Microgels, *Adv. Mater.*, 2008, **20**, 1666–1670.
- 22 E. Filippo, A. Serra and D. Manno, Poly (vinyl alcohol) Capped Silver Nanoparticles as Localized Surface Plasmon Resonance-based Hydrogen Peroxide Sensor, *Sens. Actuators, B*, 2009, **138**, 625–630.
- 23 M. R. Guascito, E. Filippo, C. Malitesta, D. Manno, A. Serra and A. Turco, A New Amperometric Nanostructured Sensor for the Analytical Determination of Hydrogen Peroxide, *Biosens. Bioelectron.*, 2008, **24**, 1057–1063.
- 24 Y. Wang, X. Yang, J. Bai, X. Jiang and G. Fan, High Sensitivity Hydrogen Peroxide and Hydrazine Sensor Based on Silver Nanocubes with Rich {100} Facets as an Enhanced Electrochemical Sensing Platform, *Biosens. Bioelectron.*, 2013, **43**, 180–185.
- 25 J. Sophia and G. Muralidharan, Preparation of Vinyl Polymer Stabilized Silver Nanospheres for Electro-analytical Determination of H<sub>2</sub>O<sub>2</sub>, *Sens. Actuators, B*, 2014, **193**, 149–156.
- 26 Y. Dong, Y. Ma, T. Y. Zhai, F. G. Shen, Y. Zeng, H. B. Fu and J. N. Yao, Silver Nanoparticles Stabilized by Thermoresponsive Microgel Particles: Synthesis and Evidence of an Electron Donor–acceptor Effect, *Macromol. Rapid Commun.*, 2007, **28**, 2339–2345.
- 27 Y. Lu, Y. Mei, M. Ballauff and M. Drechsler, Thermosensitive Core–Shell Particles as Carrier Systems for Metallic Nanoparticles, *J. Phys. Chem. B*, 2006, **110**(9), 3930–3937.
- 28 Y. F. Gao, W. W. Xu and M. J. Serpe, Free-standing Poly (N-isopropylacrylamide) Microgel-based Etalons, *J. Mater. Chem. C*, 2014, **2**, 5878–5884.
- 29 W. T. Wu, T. Zhou and S. Q. Zhou, Tunable Photoluminescence of Ag Nanocrystals in Multiple-sensitive Hybrid Microgels, *Chem. Mater.*, 2009, **21**, 2851–2861.
- 30 G. Q. Wen, H. Luo, Y. A. H. Liang and Z. L. Jiang, Auto-catalytic Oxidization of Nanosilver and Its Application to Spectral Analysis, *Sci. Rep.*, 2014, **4**, 3990.



## Supporting Information

# Poly (N-Isopropylacrylamide)-co-(Acrylic Acid) Microgel/Ag Nanoparticle Hybrids for the Colorimetric Sensing of H<sub>2</sub>O<sub>2</sub>

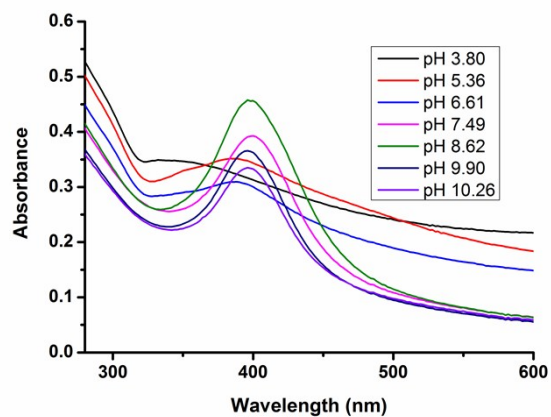
De-Man Han<sup>†,‡</sup>, Qiang Matthew Zhang<sup>†</sup>, and Michael J. Serpe<sup>\*,†</sup>

<sup>†</sup> Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

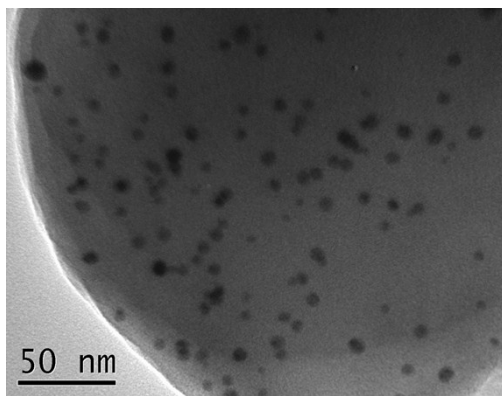
<sup>‡</sup> Department of Chemistry, Taizhou University, Taizhou, China 318000

*E-mail: michael.serpe@ualberta.ca; Fax: +1 780 492 8231; Tel: +1 780 492 5778.*

hdm@tzc.edu.cn; Fax: +86 576 88660353; Tel: +86 576 88660353

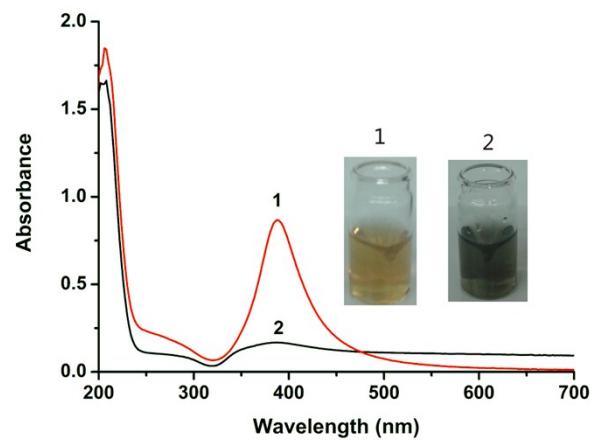


**Figure S1.** The effect of Ag NP synthesis solution pH on the optical properties of the resultant pNIPAm-co-AAc/Ag NP hybrid microgels. As can be seen, the optimal synthesis pH was 8.62.



**Figure S2.** TEM image of Ag NPs in the hybrid microgels. The microgel network contained Ag NPs with diameters of  $\sim 5$  nm.





**Figure S3.** UV-Vis absorption spectra of the Ag NPs prepared without adding pNIPAm-co-AAc microgels. 1. Fresh Ag NPs, 2. Ag NPs stored after 5 minutes.