Electronic Supplementary Material (ESI) for ChemComm.


# Lipase-Modified pH-Responsive Microgel-Based Optical Device for Triglyceride Sensing

Qiang Matthew Zhang,[a] Darren Berg,[b] Samuel M. Mugo,*[b] and Michael J. Serpe*[a]

[a]Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

[b]Physical Sciences Department, MacEwan University, Edmonton, Alberta, Canada, T5J 4S2

E-mail: michael.serpe@ualberta.ca; mugos@macewan.ca

**Experimental details**

TEM images were obtained on an ultrahigh-resolution transmission electron microscope (JEOL JEM-2010FEF) using an accelerating voltage of 200 kV. DLS measurements were carried out using a DLS/SLS-5000 compact goniometer (ALV, Langen) coupled with an ALV photon correlator.

**Microgel synthesis**

A 3-necked round bottom flask was fitted with a reflux condenser, nitrogen inlet, and temperature probe, and charged with N-isopropylacrylamide (11.9 mmol), 4-vinyl pyridine (1.38 mmol), N-acryloxysuccinimide (0.69 mmol) and BIS (0.6 mmol) in 99 mL deionized water, previously filtered through a 0.2 mm filter. The solution was purged with $N_2$ gas and allowed to heat to 70 °C for ~1 hour. The reaction was then initiated by addition of a solution of ammonium persulfate (0.2 mmol) in 1 mL of deionized water. The reaction proceeded at 70 °C for 4 hours under a blanket of nitrogen. The resulting suspension was allowed to cool overnight while stirring, and then it was filtered through a Whatman #1 paper filter in a brown glass jar.

**Preparation of lipase modified microgels**

Briefly, 0.1 g microgel was stirred overnight in 10 mL of 8 mg/mL lipase solution prepared in 0.1 M phosphate buffer (pH 7.0). Lipase was attached to the microgels via the reaction of the lipase's amine groups and the N-oxysuccinimide on the microgels. Afterward, the microgels containing the immobilized lipase were centrifuged for one hour at 4000 rpm. The residual lipase enzyme in the supernatant was determined by Lowry protein assay to determine the enzyme loaded on the microgel.

**Determination of enzyme loading by Lowry protein assay**

The mass of lipase immobilized on the microgels was determined by the Lowry method. A 0.1

mL sample (remaining lipase solution after microgel immobilization) or bovine serum albumin (1 mg/ml) standard was added to 0.1 mL 2 M NaOH. The vial was heated to 100°C for 10 min. Next, 100:1:1 (v/v/v) of 2% (w/v) $Na_2CO_3$, 1% (w/v) $CuSO_4 \bullet 5H_2O$, and 2% (w/v) sodium potassium tartrate, respectively, was added.[1] The solution mixture was left to stand at room temperature for ten minutes. Thirdly, 0.1 mL of Folin reagent was added, and the solution was left to stand for thirty minutes before reading the absorbance at 750 nm with the sample in a 1-mL cuvette. The mass of protein loaded on the microgel was determined to be the decrease in concentration from the lipase solution before and after microgel incubation with enzyme.

Calculated enzyme loading = 89 ± 5 mg/g

**Preparation of etalons**

To fabricate the Au coated coverslips (etalon under layer), 2 nm Cr and 15 nm of Au was added to a 25 x 25 mm ethanol rinsed and $N_2$ gas dried glass coverslip (Fisher's Finest, Ottawa, ON) at a rate of 1 Å $s^{-1}$, and 0.1 Å $s^{-1}$, respectively (Torr International Inc., thermal evaporation system, Model THEUPG, New Windsor, NY). The Cr/Au substrates were annealed at 250 °C for 3 h (Thermolyne muffle furnace, Ottawa, ON) and cooled to room temperature prior to microgel film deposition.

Approximately 5-10 mL of microgel solution was centrifuged at ~10000 rcf to form a pellet. The supernatant was removed and discarded, and the pellet was vortexed to loosen and homogenize the particles in the remaining solvent. A 40 μL aliquot of concentrated microgels was spread onto an annealed 25 mm x 25 mm Au-coated glass coverslip.[2] The film was allowed to dry on a 30 °C hotplate for 30 minutes before the excess microgels not bound directly to the Au layer were rinsed away with deionized water. The samples were then soaked overnight at 30 °C in a deionized water bath. The samples were then rinsed with deionized water, dried with $N_2$,

and another Au over layer (2 nm Cr for adhesion, followed by 15 nm Au) was added. The completed devices were soaked overnight in deionized water at 30 °C before spectral analysis.

**Reflectance spectroscopy**

Reflectance measurements were conducted in a specially designed sample holder using a USB2000+ spectrophotometer, a HL-2000-FHSA tungsten light source, and a R400-7-VISNIR optical fiber reflectance probe, all from Ocean Optics (Dunedin, FL). The spectra were recorded using Ocean Optics Spectra Suite Spectroscopy Software over a wavelength range of 350–1,025 nm. Measurements were performed in the sample holder, which allows for careful sample positioning, sample stability, and fine temperature control.

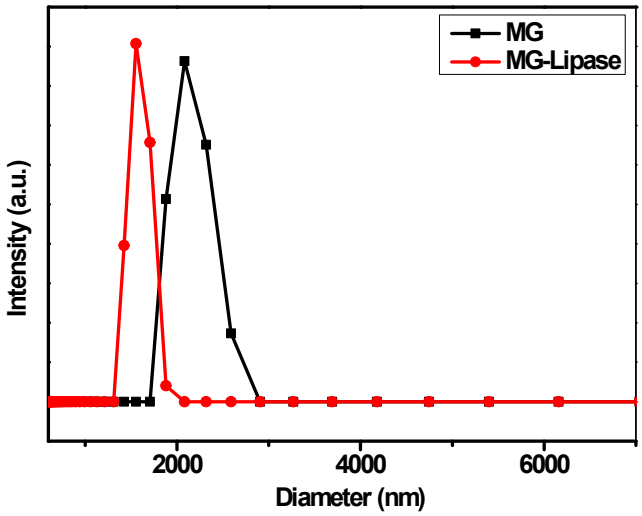


Figure S1. Microgel sizes at 30 $^{o}$C tested by dynamic light scattering (DLS).

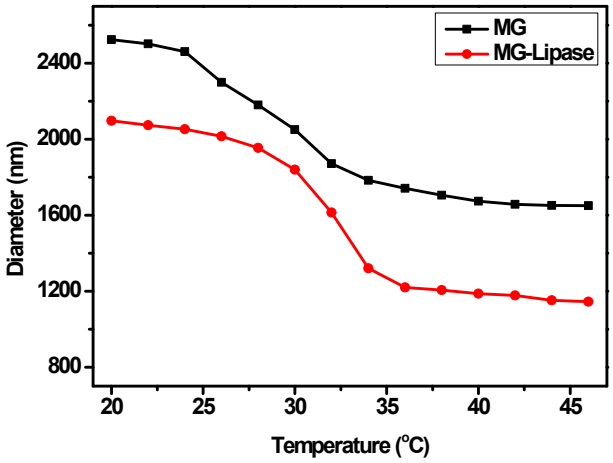


Figure S2. DLS measured microgel diameter as a function of temperature.

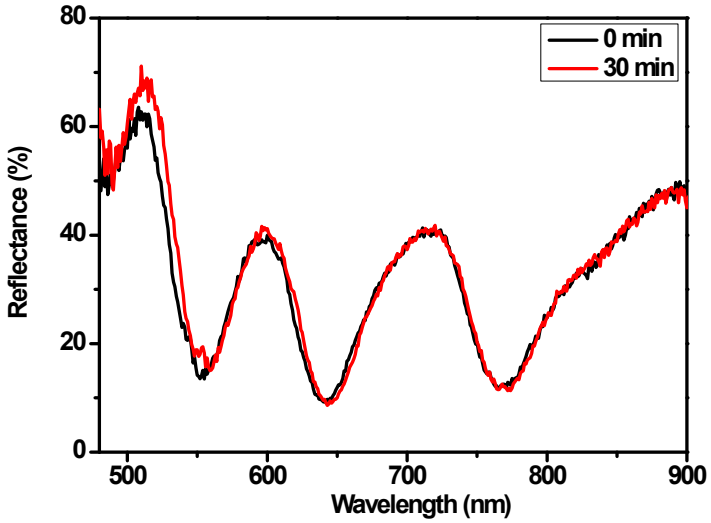


Figure S3. Reflectance spectra of etalon composed of enzyme free microgels after exposure to triolein at 30 $^{o}$C.

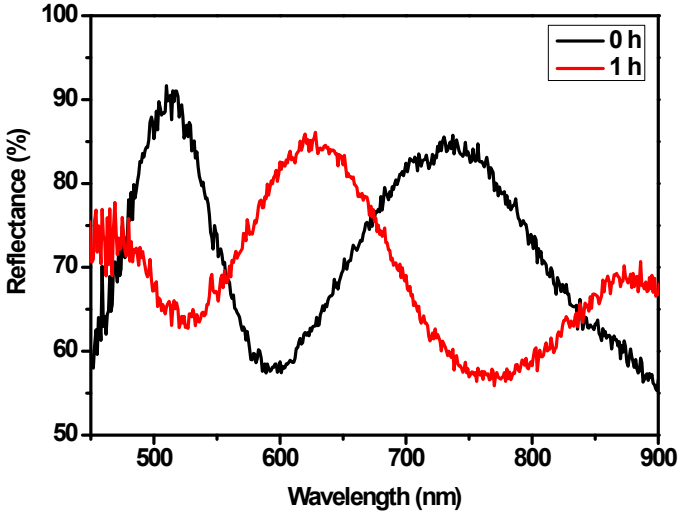


Figure S4. Reflectance spectra of an etalon composed of pyridine-modified microgels after exposure to the fatty acid $C_{18}H_{37}COOH$ (2 mg/mL final solution concentration) at 30 $^{o}$C.

1. S. M. Mugo and K. Ayton, *J. Am. Oil Chem. Soc.*, 2013, **90**, 65-72.
2. C. D. Sorrell and M. J. Serpe, *Adv. Mater.*, 2011, **23**, 4088-4092.