# Supporting Information

# Water-in-Silicone Oil Emulsion Stabilizing Surfactants Formed From Native Albumin and α,ω-Triethoxysilylpropyl-Polydimethylsiloxane

*Paul M. Zelisko[†‡]*, Kulwinder K. Flora[†§], John D. Brennan[†], and Michael A. Brook[†]***

[†]McMaster University, Department of Chemistry 1280 Main Street West,

Hamilton, Ontario, Canada L8S 4M1

[‡]Brock University, Department of Chemistry, 500 Glenridge Avenue,

St. Catharines, Ontario, Canada L2S 3A1

E-mail: mabrook@mcmaster.ca and pzelisko@brocku.ca

*To whom correspondence may be addressed at: Current address: Brock University, Department of Chemistry, 500 Glenridge Avenue, St. Catharines, Ontario L2S 3A1 Tel: +1-905-688-5550 ext. 4389; Fax: +1-905-682-9020; E-mail: pzelisko@brocku.ca

**To whom correspondence may be addressed at McMaster University Department of Chemistry 1280 Main Street West Hamilton, Ontario, Canada L8S 4M1 Tel: +1-905-525-9140 ext. 23483; Fax: +1-905-522-2509; E-mail: mabrook@mcmaster.ca

[§]Current address: HORIBA Jobin Yvon IBH Ltd., SkyPark 5, 45 Finnieston Street, Glasgow G3 8JU, United Kingdom, kulwinder.sagoo@ibh.co.uk

**5-BMF-labeled HSA.** 5-BMF (100 mg, 0.257 mol) was dissolved in DMF (10.0 mL). A 5 mL round-bottomed flask was charged with HSA (0.0141 g, 2.07 x $10^{-7}$ mol) that was subsequently dissolved in sodium bicarbonate buffer (1.0 mL, 0.5 M, pH 8.5). The 5-BMF solution (100 μL) was added to the protein solution and stirred at room temperature for 60 min. Hydroxylamine (1.045 g, 0.015 mol) was dissolved in deionized, distilled water to give a final concentration of 210 mg/mL. A solution of 5 M NaOH was added to the hydroxylamine until a pH of 8.5 was observed. The volume was then doubled with deionized, distilled water and the solution was incubated at room temperature for 60 min. The hydroxylamine solution (0.1 mL) was then added to the 5-BMF-HSA solution to scavenge any unreacted 5-BMF. The protein solution was then passed through a 10 x 300 mm column of Sephadex G-25 equilibrated with the sodium bicarbonate buffer. The first fluorescent band to elute was the labeled HSA. The 5-BMF-HSA was stored at 4 °C until required. The 5-BMF-HSA solution obtained had concentrations of 1.99 x $10^{-4}$ mg/mL (HSA) and 3.23 x $10^{-6}$ mg/mL (5-BMF), respectively, as determined by UV/visible spectroscopy. The reaction mixture was protected from light during the procedure.

**Texas Red labeled HSA.** HSA (0.53 g, 7.79 x $10^{-6}$ mol) was added to a 25 mL round-bottomed flask and dissolved in 15.0 g of 1.0 M Tris-HCl buffer (pH 7.8). Texas Red $C_2$ maleimide (5.0 mg, 6.86 x $10^{-6}$ mol) was dissolved in 1.0 mL DMSO. The Texas Red solution was added dropwise to the HSA solution and allowed to stir for 2 h at ambient temperature. The solution of labeled protein was dialyzed against 1.0 M Tris-HCl buffer (pH 7.8) for 15 h to remove any of the unreacted probe. Since the maleimide group of the Texas Red dye will preferentially react with the Cys34 residue of the protein rather than the free amine groups, labeling of the HSA is site-specific.[1-4] At pH 7, the reaction of maleimides with thiol groups has been reported to proceed 1000 times faster than the comparable reaction with amines.[5]

**5-(Bromomethyl)fluorescein (5-BMF) labeling of glucose oxidase.** Glucose oxidase (0.11 g) was added to a 10.0 mL round bottom flask and dissolved in 5.0 g of 1.0 M Tris-HCl buffer (pH 7.8). In a

separate vessel, 5-(bromomethyl)fluorescein (10.0 mg, 0.02 mol) was dissolved in 1.0 mL of DMF and added dropwise to the glucose oxidase solution. This solution was allowed to stir for 2 h at ambient temperature.

The solution of labeled protein was dialyzed against 1.0 M Tris-HCl buffer (pH 7.8) for 15 h to remove any of the unreacted probe.

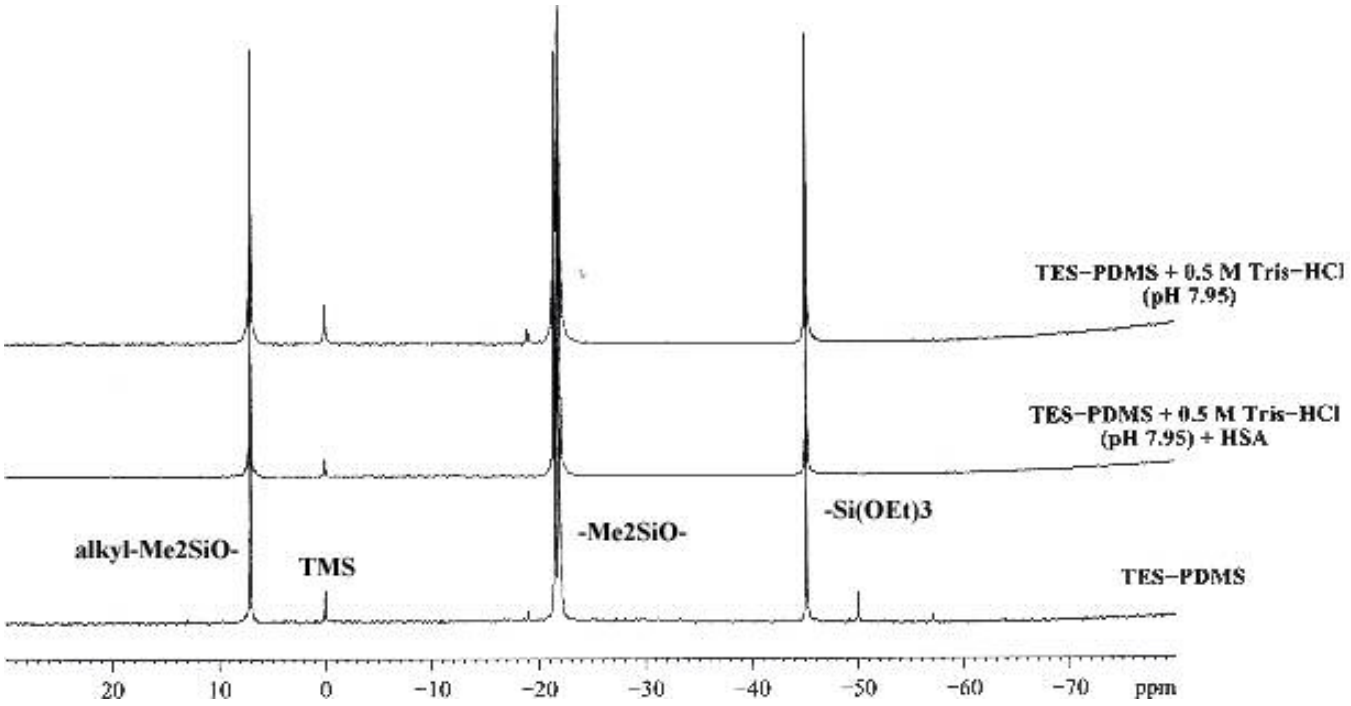


Figure 9S. $^{29}$Si-NMR spectra of TES-PMDS$_{500}$, TES-PDMS$_{500}$ exposed to 0.5 M Tris-HCl buffer (pH 7.95), and TES-PDMS$_{500}$ following exposure to 0.5 M Tris-HCl buffer (pH 7.95) and HSA. Tetramethylsilane (TMS, 1 %) was used as an internal reference.

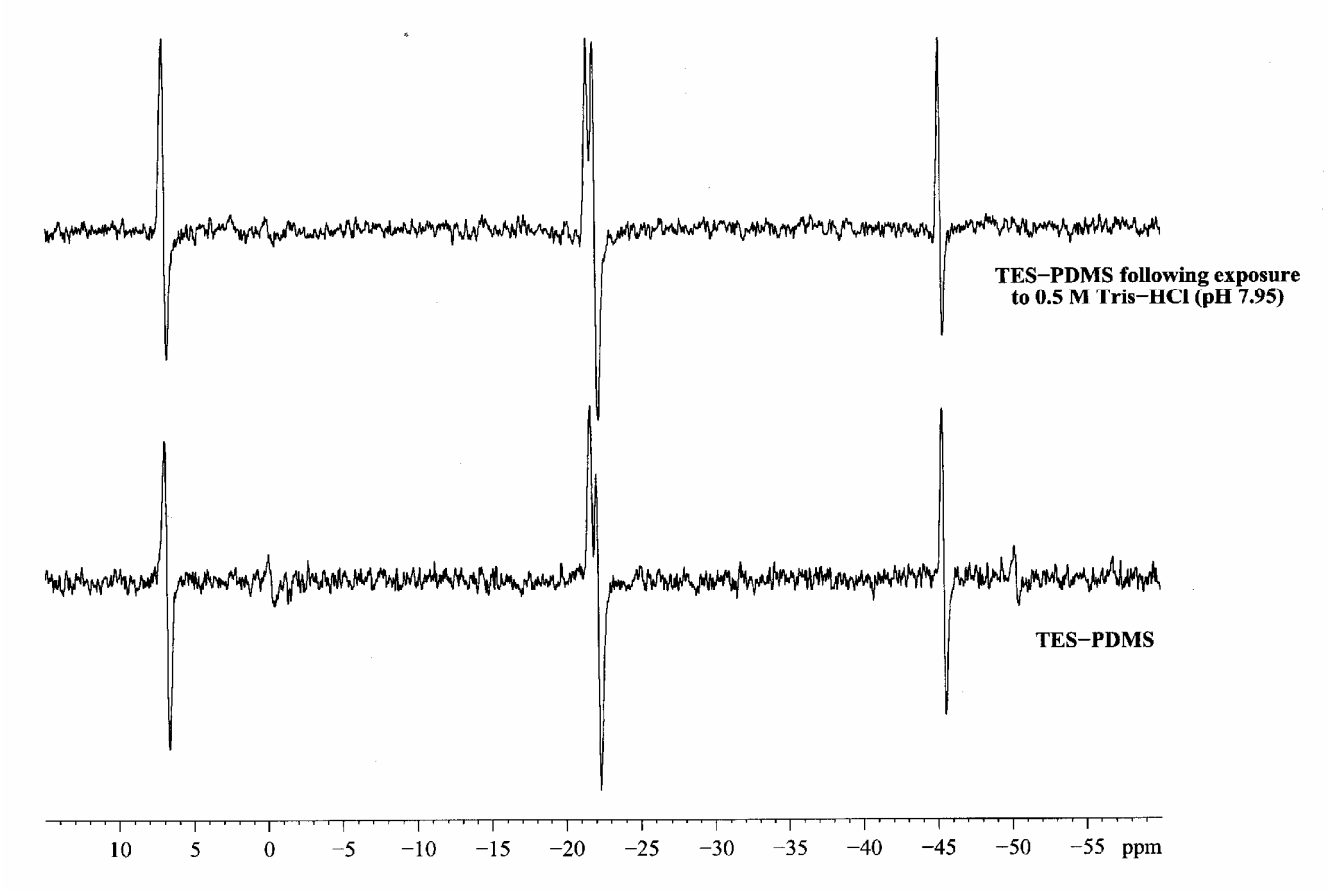


Figure 10S. $^{29}$Si INEPT spectra of TES-PMDS$_{500}$ both before and after exposure to 0.5 M Tris-HCl buffer (pH 7.95). The spectra were aquired in $CDCl_3$ with 1 % TMS and $Cr(acac)_3$.

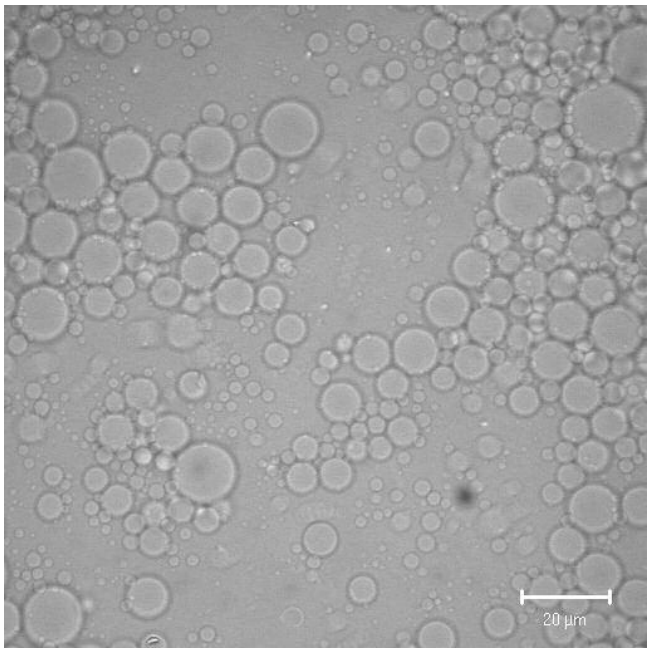


Figure 11S. Optical micrograph of an HSA/TES-PDMS emulsion containing 8 M urea as the aqueous phase taken 30 days after formulation. The scale bar represents 20 µm.

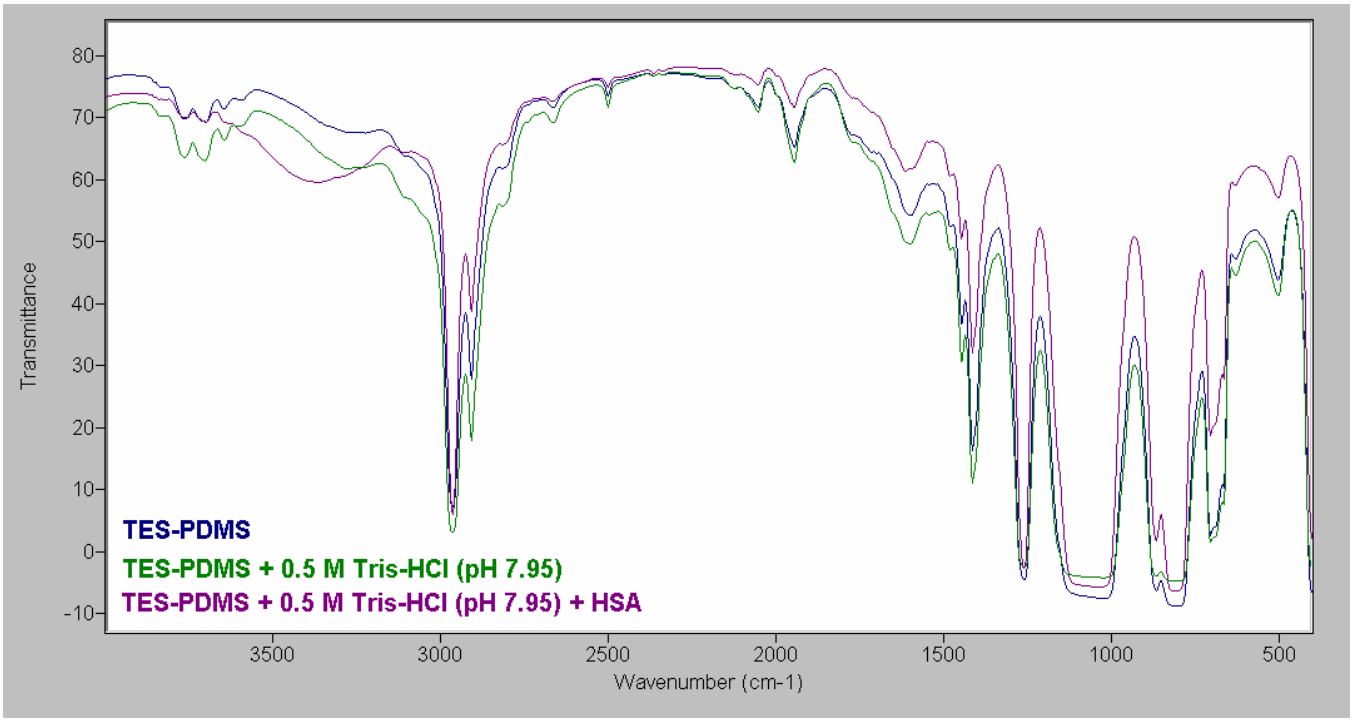


Figure 12S. Overlaid FT-IR spectra of TES-$PDMS_{28000}$ (blue), TES-$PDMS_{28000}$ following exposure to 0.5 M Tris-HCl (pH 7.95) (green), and TES-$PDMS_{28000}$ following exposure to 0.5 M Tris-HCl (pH 7.95) containing HSA (purple), demonstrating an increased intensity in the stretch at ~3300 $cm^{-1}$.

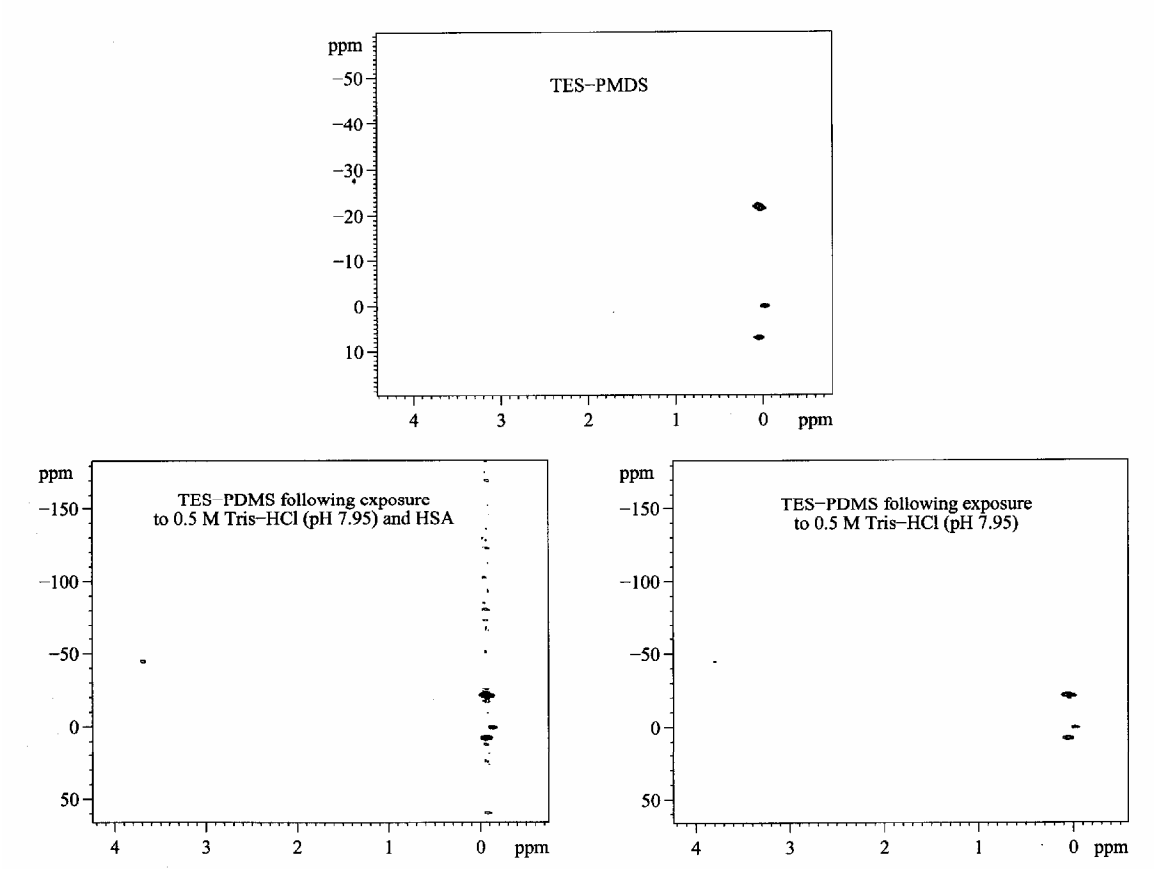


Figure 13S $^{1}H$-$^{29}Si$ HMBC spectra of TES-$PDMS_{500}$ both prior, and following exposure to 0.5 M Tris-HCl buffer (pH 7.95). The 1H-NMR spectrum is found in the F2 dimension while the $^{29}Si$ spectrum is located in the F1 dimension. The NMR spectra were conducted using $CDCl_3$ as the solvent with 1 % TMS and $Cr(acac)_3$.

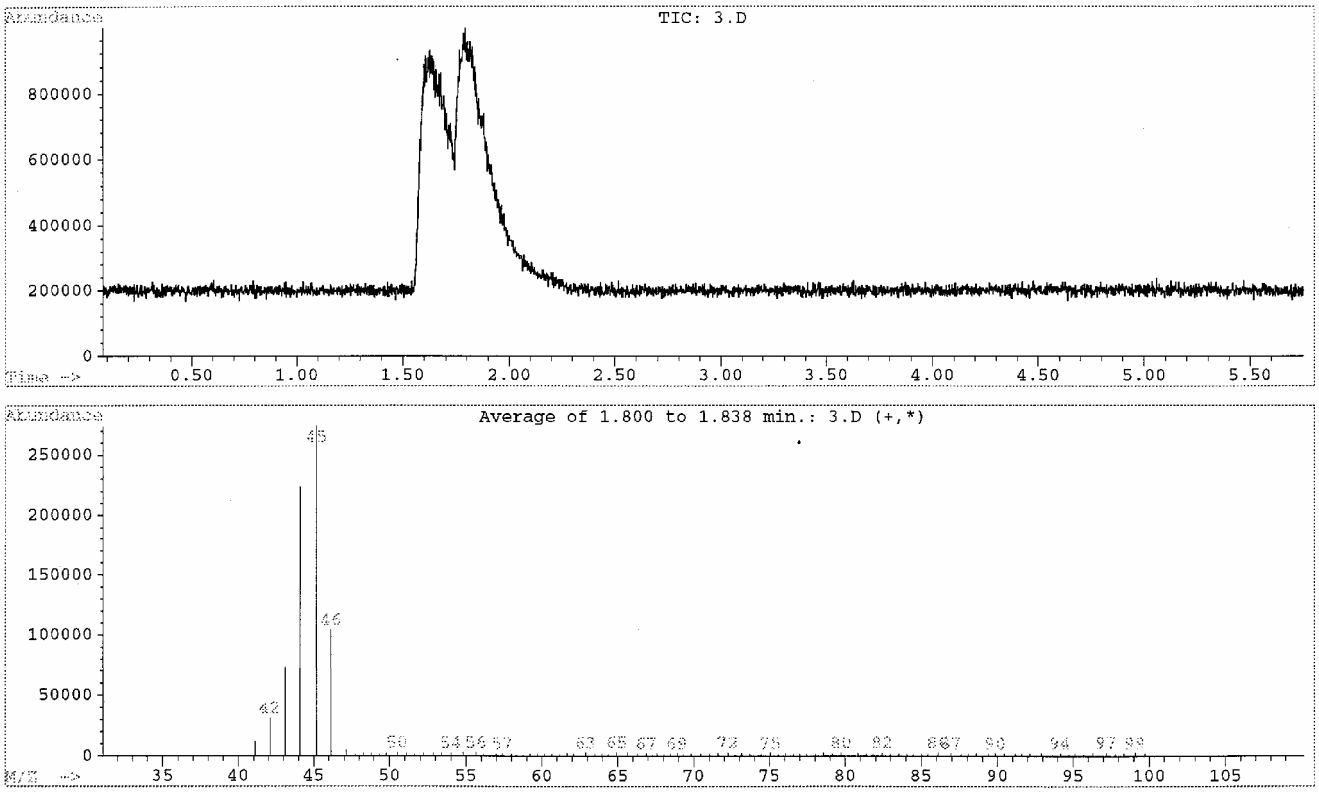


Figure 14S. The GC trace (top) and mass spectrum (bottom) of the headspace of hydrolysed TES-$PDMS_{28000}$.

Table 1S. Fragment assignments for the headspace analysis of hydrolysed TES-$PDMS_{28000}$ by GC-MS.

| Fragment | Assignment (m/z) |
|---|---|
| $M^+$ (ethanol) | 46 |
| $CH_3CH_2O^+$ | 45 |
| $H_2C{=}CH_2^+$ (loss of $H_2O$) | 28 |
| $H_2COH^+$ | 31 |
| $CO_2$ | 44 |
| $O_2$ | 32 |
| CO or $N_2$ (latter is most likely) | 28 |

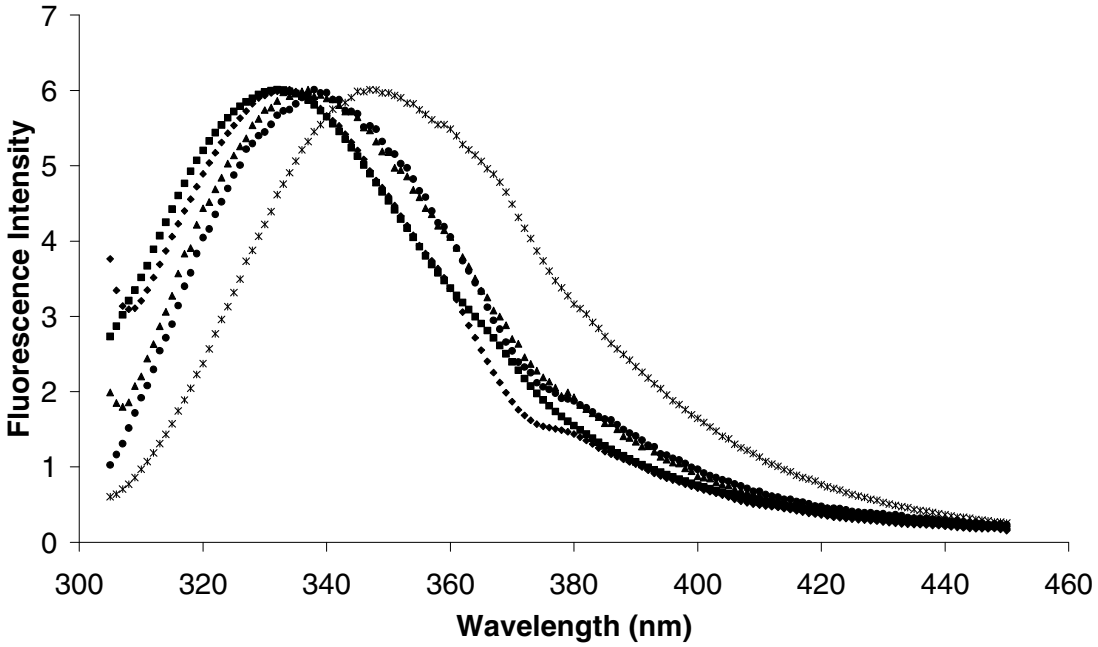


Figure 15S. Front-faced fluorescence spectra of HSA in Tris-HCl buffer (1.0 M, pH 7.8) with polarizers at 0 and 90 degrees (●), HSA in Tris-HCl buffer (1.0 M, pH 7.8) with polarizers at magic angle (▲), HSA extracted from a TES-PDMS emulsion on day 7 (■), HSA in an intact TES-PDMS emulsion on day 10 (♦), and HSA denatured by 10 M guanidine HCl (*).

**References**

[1] Heitz, J.R.; Anderson, C.D.; Anderson, B.M. *Arch. Biochem. Biophys.,* **1968**, *127*, 627-636.

[2] Smyth, D.G.; Blumenfeld, O.O.; Konigsberg, W. *Biochem. J.,* **1964**, *91*, 589-595.

[3] Gorin, G.; Martin, P.A.; Doughty, G. *Arch. Biochem. Biophys.,* **1966**, *115*, 593-597.

[4] Partis, M.D.; Griffiths, D.G.; Rogerts, G.C.; Beerchey, R.B. *J. Protein Chem.,* **1983**, *2*, 263-277.

[5] Hermanson, G.T. Bioconjugate Chemistry, 1996, San Diego: Academic Press. Chapter 2.