Electronic Supplementary Material (ESI) for Analytical Methods.


# Electronic Supplementary Information

# In Situ Hemolysis in a Three-Dimensional Paper-Based Device for Quantification of Intraerythrocytic Analytes

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## Materials and Methods

*Chemical Reagents*

We purchased Whatman chromatography paper grade 4 (GE Healthcare), 20 μm polyester mesh (SEFAR PETEX), and laminate (Fellowes) sheets from Amazon. We purchased GR Vivid plasma separation membrane from Pall Corp. We purchased 0.5 M ethylenediaminetetraacetic acid (EDTA, pH 8.0), Triton X-100, and sodium dodecyl sulfate (SDS) from Fisher Scientific. We purchased CHAPS detergent from Amresco. We purchased saponin from EMD Millipore Corporation. We purchased Drabkin's reagent and Brij 35 (30% w/w) from Ricca Chemical. We purchased lyophilized hemoglobin standard from Pointe Scientific (Canton, MI). We purchased Critoseal vinyl plastic putty from VWR. We purchased 40 mm microhematocrit capillary tubes from LW Scientific. We purchased Flexmount Select DF051521 (permeant adhesive double-faced liner) from FLEXcon (Spencer, MA). We obtained samples of whole blood from Research Blood Components (Brighton, MA). We purchased single donor, washed and packed RBCs from Innovative Research (Novi, Michigan).

## Methods

*Reference Measurement of the Hematocrit*

We measured the initial hematocrit of the whole blood sample upon arrival. We added 3 μL of whole blood to a 40 mm microhematocrit capillary tube and sealed the tube at one end with Critoseal. We centrifuged the microhematocrit capillary tubes at 1,200 RPM for 3 minutes using a ZipCombo centrifuge from LW Scientific. We obtained images of the microhematocrit tubes using an 8-bit EPSON Perfection V600 PHOTO scanner with a resolution of 800 dpi. We calculated the hematocrit of the sample by measuring the ratio of the length that RBCs occupied in the tube to the total sample length with ImageJ software.[1] We followed the same procedure for each sample with a total of n=2 replicates per measurement of hematocrit.

*Reference Method for the Quantification of Hemoglobin*

We quantified hemoglobin by adding 4 µL of whole blood to 1 mL Drabkin's reagent containing 0.05% (v/v) Brij 35. We incubated the mixture at 25 ˚C for 15 minutes and measured absorbance at 540 nm using a Varioskan LUX microplate reader. We prepared calibration curves daily using lyophilized hemoglobin standard rehydrated with di$H_2O$ (18 MΩ) and diluted over a range 3–18 g/dL. We determined the limit of detection (LOD) for the Drabkin's assay using isolated plasma (n=20).

*Determination of the Extent of Lysis in Solution*

We began surveying the efficacy of surfactants for hemolysis by adding various concentrations of liquid surfactants to liquid samples of blood. We added 1 µL of each concentration of surfactant to 40 µL of washed and packed RBCs and mixed gently. We added 10 µL of a Ficoll solution ($\rho$=1.04 g/mL) followed by 10 µL of sample to a 40 mm microhematocrit capillary tube. We sealed one end of the capillary tube with superglue and allowed it to dry for 45 minutes at room temperature. We centrifuged the microhematocrit capillary tubes at an RCF of 800 x g for 60 minutes at 4 ˚C. We obtained images of the microhematocrit tubes using an 8-bit EPSON Perfection V600 PHOTO scanner with a resolution of 800 dpi. We measured the ratio of the length of packed RBCs in the tube to the total sample length with ImageJ software.[1] We calculated the extent of hemolysis by relating the decrease in length of packed RBCs with respect to the initial length of packed RBCs in a sample of blood that experienced no hemolysis. We normalized all data to the samples of blood that experienced no hemolysis for comparison of different surfactants.

*Estimation of Sample Volume Contained in the Paper Punch*

We performed the Drabkin's assay using sample volumes of 3, 4, and 5 µL in 1 mL of Drabkin's reagent. We constructed calibration curves over the physiological range of hemoglobin (3–20 g/dL). We plotted the slope of each calibration curve against the sample volume. We fit the data using linear regression, which yielded a linear relationship between

slope and sample volume (**Fig. 2**). We used the slope obtained from calibrating our paper-based device with the same hemoglobin standards and estimated the sample volume contained within the paper punch.

*Elution Method for the Determination of Extent of Hemolysis*

We measured the extent of hemolysis in our device by quantifying hemoglobin in the end zone of the lateral channel and comparing it to the total available hemoglobin in the sample. We warmed blood samples to 37 ˚C and then added 40 µL of blood to the sample addition layer of each device. Once the sample saturated the end zone of the lateral channel we removed a 5 mm diameter circle with a standard office hole punch. We eluted the intraerythrocytic contents from the paper punch in 1.0 mL of Drabkin's reagent for 30 minutes on a rotisserie. We measured the absorbance of the sample at 540 nm using a Varioskan LUX microplate reader.

*Preparation of Hemolysate Controls*

We prepared hemolysate controls by mixing whole blood with a 50% w/v saponin solution and incubated the sample at 37˚C for 10 minutes. Next, we centrifuged the sample for 5 minutes at an RCF of 800 x g to sediment cellular membrane fragments. We removed the supernatant and quantified the hemoglobin concentration using standard methods described above.

*Fabrication of the Paper-Based Device*

We designed the hydrophobic barriers that defined the fluidic network in Adobe Illustrator and printed the layers of paper using a Xerox ColorQube 8580 printer.[2] We heated the printed layers with a Promo Heat press (PRESS-CS-15) at 280 ˚F for 45 seconds to melt the wax through the full thickness of the paper. We pretreated the paper sample addition layer with 6 µL of 50% w/v saponin in 0.5 M EDTA. We dried the treated layer at 65 ˚C for 5 minutes prior to assembly. We assembled devices using sheets of double-sided adhesive,[3] which we patterned using a Graphtex Cutting Plotter (CE6000-40). We sealed the exposed area of the second layer of paper and the blot layer with Fellowes laminate sheets to protect the user from

biological contamination. We assembled the completed device with a TruLam laminator. We used a 6 mm hole punch to pattern the plasma separation membrane.

**Figure S1**. Extent of hemolysis for (A) saponin and (B) CHAPS in solution. Various concentrations of each liquid surfactant were added to samples of blood. Extent of hemolysis was determined by measuring the distance of packed red blood cells in a microcentrifuge tube. All data is normalized to the initial sample of blood. Each data point is the mean of three replicates and the error bars represent the standard error of the mean.

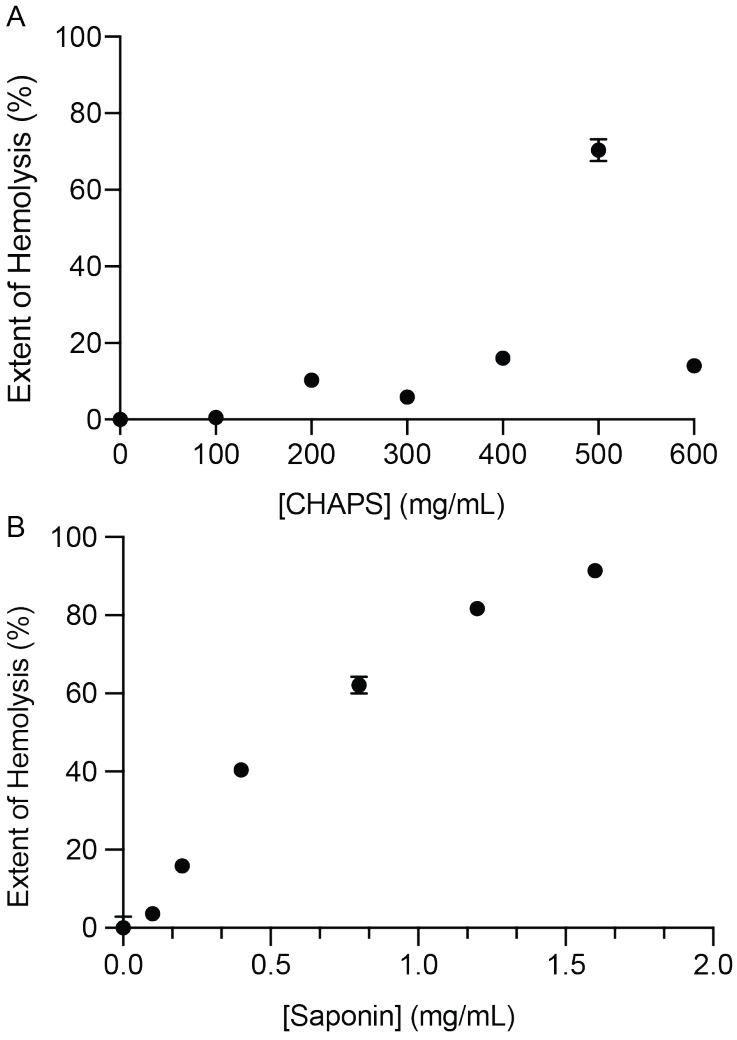

**Table S1.** Extent of hemolysis using polyester mesh as the sample addition layer.

| Hct | [Hgb] (g/dL) | Extent of Lysis |
|---|---|---|
| 35% | 4.8 | 36% |
| 55% | 8.2 | 44% |

## References

**[1]** C.A. Schneider, W.S. Rasband and K.W. Eliceiri. *Nat. Methods,* 2012, **9**, 671–675.

**[2]** E. Carrilho, A.W. Martinez and G.M. Whitesides. *Anal. Chem.,* 2009, **81**, 7091–7095.

**[3]** A.W. Martinez, S.T. Phillips, Z. Nie, C.M. Cheng, E. Carrilho, B.J. Wiley and G.M. Whitesides. *Lab Chip,* 2010, **10**, 2499–2504.