



# Phase Behavior of Hemoglobin Solutions

Kenuo Wang

e-mail: [kwang3@uh.edu](mailto:kwang3@uh.edu)

Mentor: Professor Peter Vekilov, Cullen College of Engineering, Department of Chemical and Biomolecular Engineering

Veselina Uzunova: Graduate student in the Department of Chemical and Biomolecular Engineering

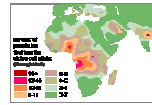
University of Houston, SURF



## BACKGROUND INFORMATION

### Sickle-cell Anemia

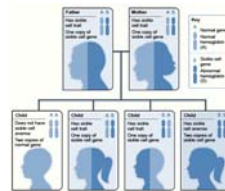
- Sickle-cell Anemia is an **inherited disease**
- Originated in Africa and Indian/Saudi Arabian subcontinent
- Caused by **mutation of hemoglobin** which results sickle-shaped red blood cells
- It can block blood vessels and cause lung tissue damage, organ damage, pain episode, blindness, and stroke



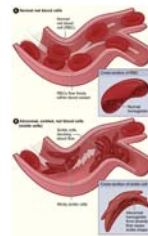
Distribution of sickle-cell anemia



Mutant hemoglobin sequence in sickle-cell patient compared to healthy people



Inheritance pattern of sickle-cell anemia



### Treatment

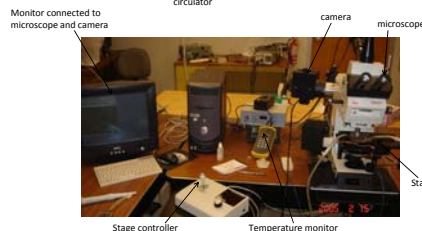
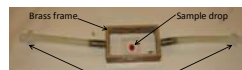
- Bone Marrow Transplant
  - The **only potential cure** for the disease
  - Expensive and has high risk, difficult to find donor
- Treatment used to avoid pain crisis and prevent complications
  - Pain relieving medications to relieve pain during sickle crisis
  - Antibiotics** can prevent infection
  - Hydroxyurea** can reduce pain crisis frequency and need for blood transfusion

### Hemoglobin Polymerization

- It cause the red blood cell's to form sickle shape
- Triggered by formation of **hemoglobin's dense phase**
- Studies have shown that **free heme concentration** can affect hemoglobin phase behavior

## METHODOLOGY

- Sample slide is glued to the brass frame
- Water circulator is used to control temperature
- Slide is observed under the microscope



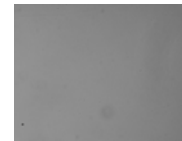
## RESULTS

### Preliminary Studies with Lysozyme

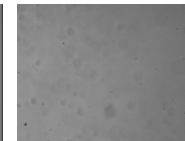
- The effect of NaCl on lysozyme phase separation
- Difference spacers are tested
  - For 5  $\mu\text{m}$  spherical spacer
  - For 1mm diameter capillary



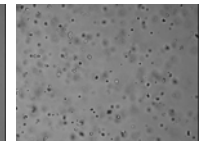
No phase separation observed



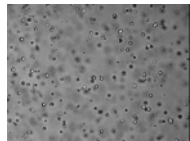
At 26.1 ° C



At 15.1 ° C



At 15.1 ° C after 2 minutes

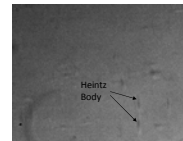


At 15.1 ° C after 3 minutes

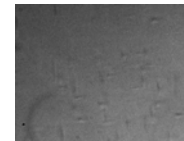
- Volume has significant effect on phase formation

### Hemoglobin Phase Separation

- The effect of PEG and heme on hemoglobin's denser phase formation
- Only PEG is added to the hemoglobin



At 25 ° C



At 29.6 ° C



At 38.7 ° C

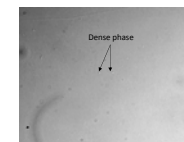


At 41.3 ° C

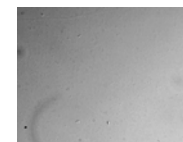
- Both PEG and heme are added



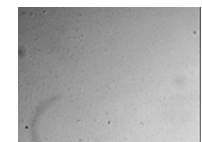
At 24.9 ° C



At 40.1 ° C



At 40.7 ° C



At 41.1 ° C

- Heme contributes to hemoglobin's dense phase formation

## SUMMARY

- Container volume affects the appearance of the dense liquid phase: phase separation is inhibited in containers narrower than 5  $\mu\text{m}$
- In the absence of heme, liquid-liquid phase separation does not occur in hemoglobin solutions; Heintz bodies of denatured protein form
- In the presence of heme and PEG, hemoglobin solutions separate into two liquid phases of, respectively, higher and lower protein concentration
- The attraction introduced by heme is crucial for the formation of the dense liquid in hemoglobin solutions
- Future works involve generating hemoglobin solutions phase diagram to quantify the result

## REFERENCE

"Sickle Cell Anemia", Medline Plus, <http://www.nlm.nih.gov/medlineplus/sicklecellanemia.html> (accessed June 23, 2009)