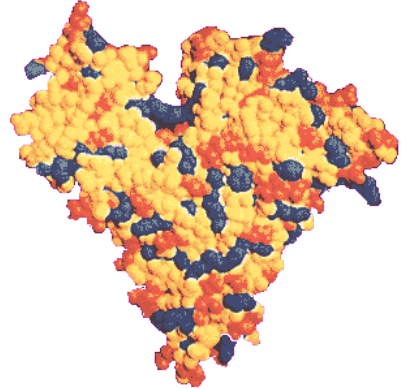


# Viscosity of aqueous bovine serum albumin

(based on the *J. Chem. Ed.* article by John Richards<sup>1</sup>)

## Purpose

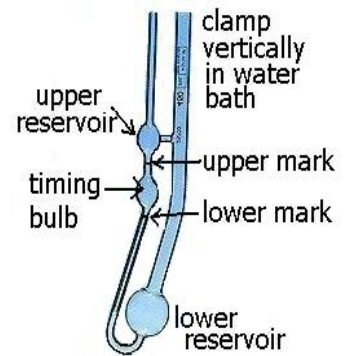
The purpose of this experiment is to measure the intrinsic viscosities of native and denatured bovine serum albumin (BSA). The intrinsic viscosities are related to protein size and shape.



## Introduction

Serum albumin, the most abundant protein in blood plasma, has been widely studied. It maintains blood pH.<sup>2</sup> Its native form was once thought to have a cigar-like shape but more recently has been described as heart-shaped.<sup>3</sup> In any case, the native form is compact. At low pH it denatures into an elongated, random coil with a much higher intrinsic viscosity. The molecular mass of BSA is 66,267 g/mol<sup>1</sup>.

Solution viscosities can be measured with an Ostwald viscometer (or "viscosimeter") and a stopwatch. The viscometer is suspended in a water bath to maintain constant temperature. The viscometer allows a controlled volume of liquid to pass through a capillary tube. Based on the Poiseuille equation, the time for the liquid to flow from the upper mark to the lower is proportional to the liquid's viscosity and inversely proportional to its density.



Ostwald-type viscometer

$$\eta/\rho = B t \quad (1)$$

The viscosity is  $\eta$ ,  $\rho$  is the mass density (not the concentration),  $t$  is the time, and  $B$  is the "viscometer constant."  $B$  is unique to a particular viscometer. It is determined experimentally by measuring the time required for pure water to pass between the viscometer's two marks. Let  $\eta_w$  denote the viscosity of pure water. The viscometer constant is calculated from

$$B = (\eta_w/\rho_w) / t \quad (2)$$

where the viscosity and density of water are known from literature.

Another type of viscometer we have in the lab is the Ubbelohde viscometer, shown at left. It differs from the Ostwald viscometer by having a third arm. When raising liquid into the upper reservoir, use a finger to close the top of the third arm.



A potassium chloride solution will be the solvent in this laboratory. Its viscosity, denoted  $\eta_o$ , is calculated from elapsed time using the equation

$$\eta_o = B \rho_o t \quad (3)$$

The "specific viscosity" is the fractional increase in viscosity due to a solute (BSA).

$$\eta_{sp} \equiv (\eta - \eta_o) / \eta_o \quad (4)$$

The small-concentration limit of specific viscosity divided by concentration is the "intrinsic viscosity",  $[\eta]$ .

$$[\eta] = \lim ( \eta_{sp}/c ) \text{ as } c \rightarrow 0 \quad (5)$$

The SI unit of  $\rho$  is  $\text{kg}/\text{m}^3$  and  $\eta$  has SI units  $\text{kg}\cdot\text{m}^{-1}\cdot\text{s}^{-1}$ , or Pa·s. The cgs unit of viscosity, Poise, has often been used. One Poise is one  $\text{g}\cdot\text{cm}^{-1}\cdot\text{s}^{-1}$ , 1 Poise = 0.1 Pa·s. The concentration is given in  $\text{g}/\text{mL}$  or  $\text{kg}/\text{m}^3$ , so intrinsic viscosity has units of  $\text{m}^3/\text{kg}$  or  $\text{cm}^3/\text{g}$ , which are related by a factor of 1000.

Richards showed<sup>1</sup> how an effective spherical radius is related to the intrinsic viscosity. In equation 6,  $N_A$  is Avogadro's number,  $M$  is the molecular mass, and  $R_e$  is the effective spherical radius of the solute. Solving equation 6 for  $R_e$  allows one to calculate effective spherical radius.

$$[\eta] = 10 \pi N_A R_e^3 / (3 M) \quad (6)$$

### Reagents and Supplies

#### Reagents in the lab

- KCl. Each group requires less than one gram.
- bovine serum albumin (BSA) stored in the refrigerator. Each group requires 3 grams.
- 1M HCl. Each group needs a few mL.
- acetone sufficient for rinsing the viscometer.
- dilute sodium hypochlorite solution (household bleach) for cleaning viscometer

#### Check out from the stockroom

- 1 500mL volumetric flask
- 2 100mL volumetric flasks
- 3 25mL volumetric flasks
- 3 50mL volumetric flasks
- 10-mL transfer pipette
- 5-mL transfer pipette
- 2-mL transfer pipette
- rubber bulb for pipettes and viscometer

#### Available in the physical chemistry laboratory

- Ostwald-type viscometer
- water bath at 25°
- magnetic stirrer and stir bar
- pH meter and electrode
- pH 7 and 4 buffers to calibrate the pH meter
- millipore filter apparatus and filters
- stopwatch

## Laboratory Procedure

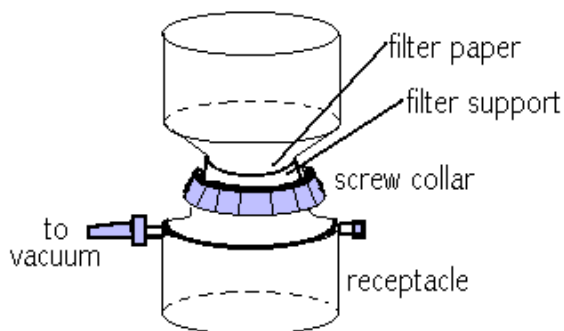
This experiment will require two laboratory periods. During the first period, prepare the native BSA solution and its dilutions, and measure their viscosities. Also prepare the denatured BSA stock solution. The remaining dilutions and viscosity measurements can be done during either the first or second lab period, as time permits. You can store one or both BSA stock solutions in the refrigerator for the second lab period. When you take a solution out of the refrigerator, vacuum filter it and warm it to room temperature before preparing dilutions.

### Prepare Solutions

Prepare 500 mL of 0.010 M KCl solution. 500 mL of 0.010 M KCl requires  $5 \times 10^{-3}$  mol or 0.373 grams. This KCl solution is the solvent for the BSA solutions.

Solutions of BSA are prone to forming foam. To minimize foaming, stir and pour gently.

Prepare 100 mL of 0.0300 g/mL native BSA stock solution in the KCl solvent. Use a beaker on a stir plate to dissolve the BSA in about 80 mL of KCl solvent. Gentle magnetic stirring will aid dissolution. The slow pace of dissolution requires patience. The stock solution should be vacuum filtered with a Millipore filter before final dilution to 100 mL in a volumetric flask. The solution tends to foam in the receptacle as it degasses under vacuum. To minimize foaming, fill and cap the upper reservoir before applying the vacuum and use one of the rubber stoppers to control the vacuum and flow. After filtering, transfer the BSA solution to a 100-mL volumetric flask and dilute to the mark with KCl stock solution.



Millipore Filter Apparatus

Prepare three dilutions of the native BSA stock solution in 25mL volumetric flasks.

- 15 mL native BSA plus KCl solvent to make 25 mL; 0.0180 g/mL.
- 10 mL native BSA plus KCl solvent to make 25 mL; 0.0120 g/mL.
- 7 mL native BSA plus KCl solvent to make 25 mL; 0.0084 g/mL.

Calibrate the pH meter with pH 7 and 4 buffers. To calibrate the Model 103 meter:

- Attach pH electrode (with internal reference electrode)
- Press "CAL". Use "^" to select "7-4" range. Press "yes".
- A dim "7" will light up. Put the electrode in the pH 7 buffer. When the reading is stable, "ready" lights up. Press "yes."
- Repeat for pH 4 buffer.

Adjust the pH of your remaining KCl stock solution to 3.0 by adding HCl dropwise. Of course, this acidic KCl solution can no longer be mixed with native BSA, so it is important that the native BSA solutions be prepared before acidifying the remaining KCl.

Prepare 100 mL of 0.01200 g/mL, pH 3, denatured BSA stock solution. Make this by pipetting 40 mL of native BSA stock solution into a beaker. Then add 50 mL KCl stock solution. The total volume must be less than 100 mL. Put the pH probe into the solution and stir gently. Add 1 M HCl dropwise to bring the pH to 3, as measured with the pH meter. Pour the denatured BSA solution into a 100 mL volumetric flask. Top off the flask with KCl stock solution.

Prepare three dilutions of the denatured BSA stock solution in 50mL volumetric flasks.

- 30 mL denatured BSA plus KCl solvent to make 50 mL
- 20 mL denatured BSA plus KCl solvent to make 50 mL
- 15 mL denatured BSA plus KCl solvent to make 50 mL

Because both the denatured BSA stock solution and the KCl are at pH 3, the diluted BSA solutions should also be at pH 3. You could check to see that all denatured BSA solutions have the same pH  $\approx$  3.

### **Calibrate the Viscometer**

Use water at 25°C. The viscosity of water at 25°C is 8.949 mPoise<sup>5</sup> = 8.949×10<sup>-4</sup> Pa·s. The density of water at 25°C is 0.9971 g/mL.<sup>6</sup> To use the viscometer, use a pipette to deliver 10 mL to the lower reservoir. That will make the lower reservoir about half full. Then use a rubber bulb to draw water up through the capillary until the timing bulb is full and the upper reservoir is partly full. Remove the rubber bulb. When the water surface reaches the upper mark, start the stopwatch. Stop the stopwatch when the water surface reaches the lower mark. Record the elapsed time. Repeat three times. (Do not refill for each trial, just draw the same liquid back up from the lower reservoir to the upper.)

Calculate the viscometer constant, B, from each of your elapsed-time measurements. Calculate the standard deviation as an estimate of the uncertainty in B.

### **Measure the Viscosity of the KCl Solvent Solution**

Pipette 10 mL of KCl solvent solution into the viscometer and clamp it in the water bath. Allow 5 minutes for temperature equilibration. Then measure the flow time. Make at least three trials. If any of the trials differ from the mean by more than 1%, make additional trials until a set of three times within 1% is obtained. The viscosity of KCl,  $\eta_0$ , is used to calculate specific viscosities of all BSA solutions so a good value of  $\eta_0$  is important to this experiment.

The density of 0.01 M KCl at 25°C is 0.99751 g/mL.<sup>8</sup> Calculate the viscosity of the KCl solvent,  $\eta_0$ . Also calculate the standard deviation in  $\eta_0$  as a measure of its uncertainty.

### **Measure Viscosities of BSA Solutions**

For each of the four native BSA solutions (stock plus three dilutions) measure the viscosity as follows. Charge the viscometer with 10 mL of solution. Allow the viscometer and solution to equilibrate in the water bath for 5 minutes. Record three flow times.

To save time, we will not measure the densities of the BSA solutions, but will assume that they are equal to the density of the KCl solvent. The error due to this assumption is probably within the uncertainty due to time measurements.

Follow Richards' instructions<sup>1</sup> for cleaning the viscometer between solutions:

"When changing solutions, the viscometer should be cleaned and dried using water, soap, water, distilled water, and acetone in that order. Use an aspirator with a short piece of tubing to speed the addition and removal of liquids."

After your last use of the viscometer, add a bleach/distilled water rinse.

Use the same method to measure the viscosities of the four denatured BSA solutions.

## Calculations

Calculate your actual BSA concentrations based on the mass you used. That is, you need not have used *exactly* 3.000 grams BSA, but if you did not you must adjust the concentrations given above accordingly. Calculating  $\eta$  requires density,  $\rho$ . Take  $\rho$  to be the density of the KCl solution: 0.99751 g/cm<sup>3</sup> for every KCl and BSA solution.

Report the following for every solution:

- concentration in grams per milliliter
- flow times
- $\eta$ ,  $\eta_{sp}$  and  $\eta_{sp}/c$

For native BSA and for denatured BSA, graph  $\eta_{sp}/c$  versus  $c$ . Perform linear regression. Include the linear regression statistics in your report. State  $[\eta]$  and its uncertainty.

Compare your intrinsic viscosities to literature values. These appear in references 1 and 7, among other places.

Use  $[\eta]$  to calculate the effective spherical radius,  $R_e$ . One hopes to find  $R_e$  much larger for the denatured protein than for the more compact native protein.

## References

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8. *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*; McGraw-Hill: New York, 1926; Volume 3, page 87. 25°C data were extrapolated from 1% to 0.075% KCl.