Aim: The detection of changes in the conformation of bovine serum albumin (BSA) by viscosity measurement and the effect of pH on the conformation of bovine serum albumin.

Principle: High concentration of urea causes unfolding ie denaturation of proteins by weakening the hydrophobic bonds that maintain the tertiary structure. This change in the protein conformation leads to a less compact molecule with a larger viscosity than the native protein. Such changes in the tertiary structure can be readily followed using an Ostwald viscometer (see figure). This , essentially consist of a capillary tube down which a known volume of protein solution is allowed to flow under gravity. The time taken for this flow is measured (t_1) and also that of the solvent (t_0); the relative viscosity is then given by-

$$\eta_{rel} = \eta_1 / \eta_0 = (t_1 / t_2) \times (\rho_1 / \rho_0)$$

where η_1 is the viscosity of the protein solution of density ρ_1 and η_0 the viscosity of the solvent of density ρ_0 . If the densities are taken to be the same then the expression simplifies to $\eta_{rel} = t_1 / t_0$

Einstein has shown that, for spherical molecules, the relative viscosity is related to the concentration of the molecule (c) and the partial specific volume (V), which is the volume occupied by the molecule and its bound water:

$$\eta_{rel} = 1 + 2.5cV$$

Materials:

Viscosity is very sensitive to temperature, so all solutions an the viscometer must be kept at $30^{O}C$ in the water bath.

1. Ostwald Viscometer
2. Water Bath at 30ºC
3. Potssium Chloride (100 mmol/liter)
4. Urea solutions (0.5, 1, 2, 3, 4, 6, and 8 mol/liter in 100 mmol/liter KCl)
5. Bovine Serum Albumin (10g/liter in 100mmol/ liter KCl and the above urea solutions)
6. Stop watch accurate to at least 0.1s

Method:

Always use the viscometer by one limb only and never squeeze the two

arms together. Rinse the viscometer with KCl solution and place it in position in water bath by carefully clamping one limb. Check that it is vertically using the plumbline and introduce exactly 20 ml (or the volume marked on the viscometer) of KCl solution at 30°C in the bulb A with a syringe or Pipette.

Leave for 5 minute to equilibrate, then either apply positive pressure to the wide limb (I) or gentle suction to the other limb (II) until the meniscus rises to upper graduation mark B. release the pressure and measure the time (to the nearest 0.1s) for the liquid to flow between the two graduation marks B and C. Repeat the experiment until the flow time agree within 0.2s and calculate the average flow time. Repeat the whole procedure 10with the urea solution alone (t_0), which are the solvents, and then with bovine serum albumin dissolved in the urea (t_1). Plot the values of t_0 and t_1 against the concentration of urea and join up the points with smooth curves. Select convenient concentration of urea and calculate the relative viscosities (t_1 / t_0) using the values from the curves. This ensures that any slight errors involved in the determination of t_1 and t_0 are not magnified on taking the ratios. Finally prepare the graphs of the relative viscosity against the concentration of urea and comment on the results. In additions , calculate the partial specific volume of serum albumin in 10 mmol/literKCl and in 8 mol/ liter urea. Assume that the molecule remains spherical so that Einstein's equation is valid.

For the determination of pH on the conformation of bovine serum albumin
Material required as above experiment and pH meter.

Method:

Using the ostwald viscometer, follow the structural change in albumin dissolved in 100 mmol/KCl and distilled water as the pH is varied over the range 2-12. Comment on the results.