PROGRESS REPORT FOR AINGRA04203

PROJECT TITLE
The effect of confinement on the nanoscale structure of hemoglobin solutions by small angle neutron scattering

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SCIENTIFIC OBJECTIVES
Previously we have established that a reductionist model of the erythrocyte (red blood cell; RBC) suitable for the interpretation of small angle neutron scattering data is a concentrated solution (~30% volume fraction) of haemoglobin represented as hard spheres, and that this solution is far from thermodynamically ideal by virtue of the self-association of the spheres. The study will examine the effect of confinement, the RBC plasma membrane, on the nanoscale-structure of concentrated solutions of free haemoglobin (Hb_4), haemoglobin with bound carbon monoxide (Hb_4-CO), and haemoglobin bound with bound oxygen (Hb_4-O2), using a combination of rheological measurements and small angle neutron scattering (SANS) on whole RBCs and concentrated solutions of cell-free haemoglobin.

PROGRESS REPORT and RESEARCH OUTCOMES
In contrast to earlier investigations, which considered suspensions of human red blood cells, here we have considered suspensions of horse red blood cells. This system has a number of positive features; it is possible to obtain sufficient samples of this material for rheological and scattering studies from a single donor without health risk; and the material is well characterized in terms of its rheology [1]. Our initial investigation focused a well understood rheological manifestation of the cell shape, the rouleaux dependent thixotropic behavior of blood, where we used the scattering from a sample under a shear rate to produce a solution of uniaxially aligned ellipsoids and make certain conclusions about the spatial relationship between hemoglobin and cell membrane [2]. To reproduce initial results on suspensions of human blood cells, and extend this work, we investigated the shear rheology of horse red blood cells with different shape cells. The different cell shapes were generated osmotically by placing cells in hypo, hyper and isotonic salt solutions. The flow curves are shown in Figures 1-3.

Fig. 1 Flow curve of hypotonic (spherical) red blood cells (80% hematocrit).
Fig. 2 Flow curve of isotonic (biconcave disk) red blood cells (82% hematocrit).

While the experimental curves show that suspension thixotropy becomes more pronounced with an increase in cell volume, it was difficult to reproducibly quantify this effect as it appears very sensitive to cell volume fraction (hematocrit). Rheological investigated on similar solutions but with cells lysed and cell membranes removed by centrifugation (confinement removed) reveals a Newtonian fluid which show very little ionic strength (salt concentration) dependence of the viscosity. This is quite consistent with a hard sphere model of horse hemoglobin.

However modeling scattering data from these hemolysates indicates a degree of aggregation of the tetrameric unit. Figure 4 shows first the reductionist model, and the calculated scattered intensity from Hayter-Penfold model including screened electrostatics [3]. Figure 5 shows experimental SANS data from a solution of horse hemoglobin.

Fig. 3 Flow curve of hypertonic (shrunken biconcave disk) red blood cells (80% hematocrit).

Fig. 4 Cartoon of the rbc intracellular solution where red disks represent hemoglobin molecules in the aqueous solution (left) and calculated differential scattered cross-section (right).
Fig. 5 Scattered intensity from carbonmonoxyated horse red blood cells at 80% cell volume in 154 mM NaCl in D$_2$O.

Attempts to measuring the scattering patterns from these cell suspensions were not successful. Unlike previous measurements, where a Couette shear cell with a 0.5 mm gap was used, this cell was not available. We were not able impose laminar flow conditions on the 1 mm gap shear cells and subsequent SANS measurements on cell suspensions were highly irreproducible.

Time was not sufficient to consider the effect of oxygenation on the rheological properties or scattering curves of horse blood or lysates.

Conclusions

Horse blood cells are an appropriate system to consider the effects of confinement on the nanoscale arrangement of hemoglobin inside red blood cells by scattering and rheological investigations. Furthermore it is possible inhibit the formation of rouleaux by changing the shape of cells. This opens the opportunity for studying the aligned cells in the absence of the cell distortions caused by high shear rates.

References:

