FT-IR and Raman Microspectroscopic Analysis of Red Blood Cells

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Adult erythrocytes and nucleated erythrocyte progenitor cells (reticulocytes), collectively called the erythron, provide an excellent model to investigate the application of vibrational spectroscopy to whole cell analysis. There are several advantages in working with peripheral blood erythrocytes compared to other cell types. These include simple sample preparation because erythrocytes are readily isolated from whole blood with minimal contamination from other cells. Furthermore peripheral blood erythrocytes from a normal human adult are fully mature ensuring a relatively homogenous differentiated cell population. Finally, because of the high concentration of hemoglobin (Hb) in a single adult erythrocyte (22 M) Raman spectra of single living erythrocytes can be obtained using a water immersion objective and 632.8 nm excitation.

Three projects investigating the vibrational spectroscopy of red blood cells will be presented.

One investigates a hypothesis put forward by M. Diem et al.[1] that the nucleus within a cell can be too compact to transmit IR radiation. To test this hypothesis spectra of intact avian erythrocytes (containing nuclei) were compared with spectra of normal adult erythrocytes (no nuclei) and also spectra of lysed avian erythrocytes (where the nuclei were exposed and had also increased in size). The spectra of chicken and human erythrocytes were essentially identical, however, the spectra of lysed erythrocytes with intact „swollen“ nuclei from avian erythrocytes exhibited very intense symmetric and asymmetric phosphate stretching bands compared to the intact chicken cells. The result provides indirect support for the Diem hypothesis.

The second project combines FT-IR spectroscopy with partial least squares (PLS) to determine carbon monoxide concentration in blood. The advantage of FT-IR spectroscopy compared to the more conventional UV spectroscopy for CO concentration determination is that the former technique can be used on dried blood. PLS regression analysis using leverage correction as the validation method was performed on second derivative spectra for a range of erythrocyte CO concentrations that were prepared by mixing various proportions of deoxygenated and carboxylated erythrocytes. A regression coefficient of 0.996 was obtained in the spectral range 3646-706 cm^{-1}. An investigation of the regression weightings revealed that the CO stretching vibration and the amide II band were strongly correlated. When a PLS validation on only the CO stretching region (2000-1930 cm^{-1}) was modeled a value of R = 0.983 was achieved. Recalculating the regression coefficient including the CO stretching region and the amide I and II region (2000-1476 cm^{-1}) produced a coefficient of R = 0.996. When only the amide I and II region (1740-1476 cm^{-1}) was modeled a value of R = 0.923 was obtained. This rather surprising result infers that conformational change associated with the R to T state transition can be detected with FT-IR spectroscopy and moreover the inclusion of the protein modes in the PLS validation model enhances the quantitative determination of CO concentration in blood.

The third project entails the micro-Raman characterisation of single living erythrocytes using 632.8 nm excitation and explores the diagnostic potential of single cell analysis using this technique in combination with multivariate methods. In particular we demonstrate the potential of the technique to distinguish between oxygenated and deoxygenated cells, foetal and adult erythrocytes, and sickle cell trait and normal adult erythrocytes. The wealth of structural detail on single living cells using 632.8 nm excitation relates mainly to perturbations of the porphyrin macrocycle induced by globin conformational changes, ligand exchange, oxidation and spin state changes associated with the Fe centre.

[1]Boydston-White S. Gopen T. Houser S. Bargonetti J. Diem M. Infrared spectroscopy of human tissue. V. Infrared spectroscopic studies of myeloid leukemia (ML-1) cells at different phases of the cell cycle. [Article] Biospectroscopy. 5(4):219-227, 1999.