Accurate de-oxygenation of *ex vivo* whole blood using sodium Dithionite.

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Introduction:
In order to determine the effect of oxygen saturation on transverse relaxation rate, an accurate method for the de-oxygenation of ex *vivo* whole blood is required. Sodium dithionite ($Na_2O_4S_2$) has been used to induce dissociation of dioxygen from oxyhemoglobin of intact erythrocytes during calorimetric studies of oxyhemoglobin dissociation [1]. Dithionite induces dissociation of dioxygen by the reduction of the external dioxygen and not by diffusion into the cell. As a result, the structure and function of the cell membrane remains relatively unchanged. At low dithionite concentrations (<15 mM), the reduction of oxyhemoglobin is reversible and the by-products of the oxidation of dithionite (sulfite, sulfate and thiosulfates) do not effect the structure or nature of the endogenous proteins [1].
The purpose of this study was to determine the correlation between concentration of dithionite added and the oxygen saturation of whole human blood. The rate of re-oxygenation was also assessed by measuring the oxygen saturation of spiked blood samples over a one hour time period after the addition of dithionite. In order to verify that the addition of dithionite at low concentrations does effect the relaxation properties of plasma and blood, the T1 of plasma and blood samples were measured at 0.47 T prior to and after the addition of dithionite.

Materials and Methods:
BLOOD SAMPLES: Heparanized whole human blood was obtained from a local blood bank and all experiments were performed within six hours after the blood was drawn. The blood contained sodium heparin as the anti-coagulant.
CORRELATION STUDIES: Blood was fractionated into six 10-g samples. Dithionite was added so that concentration ranged from 0 to 5 mg dithionite/g blood. The percent oxygenation of all samples were measured using an automatic blood pH/gas analyzer (AVL 995) immediately prior to the addition of dithionite as well as after 1, 15, 30, 45 and 60 minutes after mixing. A calibration curve was generated by plotting the percent oxygenation 30 minutes after mixing versus the concentration of dithionite added. The goodness of fit (back-calculated values) of the spiked blood samples was evaluated by preparing five additional blood samples spiked with known concentrations of dithionite giving samples with theoretical oxygenation values of 0, 24, 48, 62 and 70% O_2. The percent oxygenation was then estimated from the calibration curve based on the concentration of dithionite added. The mean percent of the theoretical value (percent recovery) of the O2% was then determined for each of the five blood samples. The rate of re-oxygenation was evaluated by determining the relative increase in the percent oxygen as a function of time after the addition of dithionite.
RELAXATION RATES: All longitudinal relaxation rates (R1, s^{-1}) were determined using a BRUKER Minispec operating at 0.47 T and 37° C. R1 values were obtained using an Inversion Recovery sequence with 25 different inversion times. Samples were prepared at dithionite concentrations of 1.2 and 2.5 mg dithionite/g blood.

Results And Discussion:
CORRELATION STUDIES: Figure 1 shows the correlation between the percent oxygen and the concentration of dithionite added 30 minutes after mixing. Full de-oxygenation was observed at concentrations over 2.5 mg dithionite/g blood.

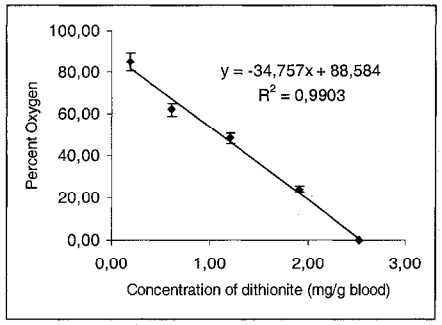

***Figure 1**: Correlation between concentration of dithionite and the measured oxygenation.*

A linear relationship between the concentration of dithionite added and the oxygenation was observed 30 minutes after mixing. The accuracy of this method, as described by the percent recovery values, is summarized in Table 1 below.

***Table 1**: Comparison of the theoretical O_2% values obtained based on the calibration curve and the measured values.*

Conc. (mg /g blood)	Theoretical O_2%	Measured O_2%	Recovery %
2.50	0	0	100
1.90	24.1	21.0	87
1.20	48.2	47.8	99
0.61	62.1	60.5	97
0.53	70.1	71.5	102

Table 2 shows the change in the oxygenation as a function of time. At low concentrations of dithionite (< 2.5 mg/g) re-oxygenation begins within the first 25 minutes after mixing. However, the oxygenation remains relatively constant within a time interval of 30 to 45 minutes after mixing.

***Table 2**: Change in O_2% as a function of time.*

Conc. mg/g	O_2% 1 min.	O_2% 15 min.	O_2% 30 min.	O_2% 45 min.	O_2% 60 min.
2.5	0	0	0	0	5.1
0.61	40.3	57.7	62.1	60.1	61.4
0.19	77.9	78.1	85.0	84.9	85.9

RELAXATION RATES: No significant change (p=0.02) was observed in the relaxation rates of blood or plasma after the addition of dithionite.

Conclusions: Sodium dithionite can be used to accurately de-oxygenate whole human blood. A linear relationship exists between the concentration of dithionite added and the percent oxygenation 30 minutes after the addition of dithionite. The addition of dithionite at concentrations less than or equal to 2.5 mg/g blood does not effect the longitudinal relaxation rates of either blood or plasma.

References:
1. Luciano, F., et al., Journal of Inorganic Biochemistry 23, 109-117 (1985).