CHAPTER V

DISCUSSIONS

The objects of this thesis concentrated on the investigation of oxygen consumption in red blood cells using nuclear magnetic resonance techniques (NMR). The NMR techniques using in this experiment were relaxation times, namely spin-lattice relaxation time (T1) and spin-spin relaxation time (T2). Due to dissolved oxygen was one of the paramagnetic substances that could effect to the relaxation time change, the relationship between proton spin-lattice and spin-spin relaxation time change and the quantity of dissolved oxygen in the solutions containing different quantities of red blood cells was studied and applied for this object.

According to this object, the first experiment was the measurement T1 and T2 values of phosphate buffer saline solutions (PBS), which was used as solvent of red blood cells. After optimized parameters for T1 and T2 measurements were determined by the method as described in chapter III, both measurements were applied for phosphate buffer saline solutions which was divided into two parts, one was degassing oxygen from the solution and the other was oxygen containing solution and the results were shown in table 4-1 and 4-2. Because of the some variation of T1 and T2 values of each measurement, the average values were used in data analysis. The The sample standard deviations were obtained using statistic calculation programme.

From the data in table 4-1 and 4-2, the average T1 value and T2 values of PBS solution with degassing oxygen was larger than the average T1 value of PBS solution without degassing oxygen while the average T2 values of both samples were not different. The results could be concluded that only spin-lattice relaxation time was oxygen-dependent and the increasing of the T1 values varied with the quantity of dissolved oxygen in solution.

To study the relationship between proton spin-lattice relaxation (T1) change and times, the dissolved oxygen solutions containing red blood cells 30%, 15%, and 7.5% by volume were measured and the results were shown in table 4-3 to 4-5. From the data 4-3 to 4-5, it showed that the trend of T1 values change were increased with times-dependent in all three concentrations and the T1 values at 30% red blod cells were larger than the ones at 15% red blood cells and 7.5% red blood cells respectively. Linear-regression analysis was used to present the trend of the average T1 values change (second) of three concentrations of dissolved oxygen containing red blood cells solutions, varied with the measured times (min.) (figure 4-1). Accordingly, it could be concluded that all three concentrations of red blood cell solutions showed the significant time-dependent increasing in the T1 values. However, the data in table 4-6 to 4-8 also showed that time-independent of T2 relaxation time change. The average T1 and T2 values were obtained using the statistic calculation programme and were summarized in table 4-10 and 4-11. The average T1 and T2 values were used in the experiment because T1 values were varied with the rate of consuming of oxygen by red blood cells. From the supposing that the the highest T1 values of PBS solution with degassing oxygen was relative to 100% by volume of dissolved oxygen and the lowest T1 of PBS solution without degassing oxygen was relative to 0% by volume of dissolved oxygen, the percentage of oxygen consumption of red blood cells at different times were calculated by using the equation followed and presented in table 4-11 :

$$(T1_{RBC} - T1_{min}) \times 100 / (T1_{max} - T1_{min}) \qquad (5\text{-}1)$$

where $T1_{RBC}$ = the T1 values of dissolved oxygen solutions containing each concentration of red blood cells (30%, 15% and 7.5%) (sec.)

$T1_{max}$ = the T1 values of PBS solution with degassing oxygen (sec.)

$T1_{min}$ = the T1 values of PBS solution without degassing oxygen (sec.)

From the data in table 4-11, it showed that the percentage of dissolved oxygen which red blood cells consuming at 30% of red blood cells was larger than the lower concentrations. Accordingly, it concluded that the quantity of oxygen which was consumed by red blood cells depended on the quantity of red blood cells.

The average T1 values and the percentage of oxygen consumption by red blood cells of three concentrations could not take to compare because of the different of red blood cells. Accordingly, both average T1 values and the quantity of oxygen consumption of red blood cells were adjusted to be equal to the same unit volume by reciprocal to the percentage of three concentrations of red blood cells. To evaluate the relationship between the percentage of oxygen consumption of red blood cells per unit volume of red blood cells and the percentage of oxygen consumption per unit volume of red blood cells, the linear-regression analysis was used (data in table 4-11) and the relationship was presented in figure 4-2. From figure 4-2, it showed that the trend of the change of T1 values dependent on the increasing of the percentage oxygen consumption of red blood cells and this relationship could demonstrated in the form of linear-regression equation as show bellow:

$$Y = -4.674x10^{-4} + 9.842x10^{-3}X \qquad (5\text{-}2)$$

$$r = 0.999172$$

where Y = the differential between average T1 value of dissolved oxygen solution containing red blood cells and T1 of PBS solution without degassing oxygen per unit volume of red blood cells (sec.)

X = the percentage oxygen consumption of red blood cells per unit volume of red blood cells (%)

r = the regression coefficient.

The regression coefficient (r) was approach to the value of 1, therefore, it demonstrated that the trend of the increasing of oxygen consumption could be the linear relationship. These results concluded that the rate of oxygen consumption of red blood cells were linear relationship.

From equation (5-2), the rate of oxygen consumption of red blood cells could be determined. On expectation that if dissolved oxygen could be effect to the T1, T1 should be increased and finally constant at the exact values and these values should be equal to the T1 PBS solution with degassing oxygen. On the other hand, most T1 values of all concentrations of red blood cells at 120 minutes were lower than the T1 values of PBS solution with degassing oxygen and T1 values at 30% red blood cells were larger than the lower concentrations respectively (figure 4-3). This resulted that the red blood cells could consume the quantity of oxygen equal to the red blood cells' volume because when the amount of red blood cells increased, the T1 values also incresed. The plot of the diffence between T1 values of samples and T1 values of PBS solution against percentage of red blood cells was shown in figure 4-3 (data in table 4-10). Using linear-regression analysis, the linear-regression equation that described about this relationship was:

$$Y = 0.184 + 0.017X \qquad (5\text{-}3)$$

$$r = 0.956325$$

where Y = the diffence between T1 values of samples and T1 values of PBS solution (sec.)

X = the percentage of red blood cells (%)

r = the regression coefficient.

From the equation (5-3), the hematocrit value (HCT,%) could be determined and shown in table 4-14.

From the T2 data in table 4-6 to 4-8, the averaged T2 values at each measured times of 12 sample were calculated by using the statistic calculation programe. Due to the variation of data and all results were shown in table 4-12 including the sample standard deviations of them. The plot of rate of the T2 relaxation against percentage of red blood cells was shown in figure 4-4 using data in table 4-12. From figure 4-4, it showed that the change of rate of the T2 relaxation was decreased which vary with the increasing of the percentage of red blood cells and corresponded to the increasing of T2 values. It could be concluded that T2 values change depended on the quantity of red blood cells.

Finally, it could be concluded that the NMR techniques are one of the most powerful instrument for science and biological science. As this experimental concern, the rate of oxygen consuming by red blood cells could be determined by T1 which could be converted to oxygen consumption of red blood cells. Furthermore, The results were clearly shown that the T1 values gave the same trend of the HCT values of hematology analyzer at the concentration of 30% by volume of red blood cells and T2 values gave the same trend of the HCT values of hematology analyzer at the concentration of 7.5% by volume of red blood cells.