CHAPTER - VIII

SUMMARY AND CONCLUSIONS

SUMMARY

In this thesis, the ESR investigations are carried out to understand the reduction process, diffusion and mobility of permeable, partially permeable and impermeable nitroxyl radicals in pure water, liposomal solution and high viscous medium using multi frequency ESR spectrometers, which will be useful for optimizing the ESR/OMR imaging parameters. In addition, the DNP studies were also carried out for nitroxyl radicals in high viscous medium.

The ESR parameters such as the line width, g-factor, hyperfine coupling constant and rotational correlation time for 1 mM concentration of ^{14}N-labeled TEMPONE, TEMPO and TEMPOL in pure water were obtained. From the results, the TEMPONE radical has narrowest line width and fast tumbling motion compared with TEMPO and TEMPOL radicals. The reduction process was recorded for 1mM concentration of ^{14}N-labeled TEMPONE, TEMPO and TEMPOL in 1mM concentration of ascorbic acid as a function of time using X-band ESR spectrometer. The half-life time and decay rate were estimated for 1mM concentration of ^{14}N labeled nitroxyl radicals in 1 mM concentration of ascorbic acid. From the results, the increase in half-life time and decrease in decay rate were observed for TEMPONE compared with TEMPO and TEMPOL radicals, which indicates the higher stability of TEMPONE radical. The observed radical scavenging activity is also higher for TEMPONE radical. Hence, the TEMPONE radical can act as a good redox sensitive spin probe for in vivo/in vitro ESR and OMR imaging.

The permeable and impermeable nature of ^{14}N-labeled deuterated MC-PROXYL and carboxy-PROXYL was demonstrated using an L-band spectrometer. The liposome concentration was optimised as 300 mM for

phantom studies. The linewidth broadening was observed for nitroxyl spin probe in liposomal solution. The hyperfine coupling constant corresponding to aqueous and lipid peak was estimated. The rotational correlation time of nitroxyl spin probe in the liposomal solution indicates the less mobile nature in high viscous medium. The partition parameter reveals the permeable and impermeable nature of nitroxyl spin probe. The membrane permeability (R value) decreases with increasing concentration of liposome. The decrease in R value describes an increase of membrane permeability. This study illustrates that the ESR technique can be used to differentiate between the intra- and extra-membrane water by loading the liposomes vesicles with a lipid permeable nitroxyl spin probe.

The permeable and impermeable nature of MC-PROXYL and carboxy-PROXYL was demonstrated by using low frequency 300 MHz ESR spectrometer. The liposome concentration was optimized as 400 mM for phantom studies. The line width broadening was observed for nitroxyl spin probe in liposomal solution. The hyperfine coupling constant corresponding to aqueous and lipid peak was estimated. The rotational correlation time nitroxyl spin probe increases with increasing concentration of liposomal solution, which indicates the less mobile nature in high viscous medium. The partition parameter reveals the permeable and impermeable nature of nitroxyl spin probe. The membrane permeability (R) value decreases with increasing concentration of liposome for MC-PROXYL, which reveals the increasing nature of membrane permeability. This study illustrates that the ESR technique at low frequency can be used to differentiate between the intra- and extra-bilayer lipid membranes water by loading the liposomes vesicles with a lipid permeable nitroxyl spin probe.

The ESR parameters of the line width, signal intensity ratio, hyperfine coupling constant, and rotational correlation time were reported for 2 mM ^{14}N-

labeled TEMPONE, TEMPO, carbamoyl-PROXYL and carboxyl-PROXYL in pure water and water/glycerol mixture in the ratio of 15:85. The line width broadening increases two fold in the high-viscosity sample (85% glycerol) of ^{14}N-labeled carbamoyl-PROXYL and carboxy-PROXYL, but this effect is significantly smaller in the high-viscosity sample (85% glycerol) of ^{14}N-labeled TEMPO. The line width broadening increases by ~50% in the high-viscosity samples (85% glycerol) of ^{14}N-labeled TEMPONE. The correlation time increases (~30 times) for 2 mM ^{14}N-labeled carbamoyl-PROXYL and carboxy-PROXYL solution in 85% glycerol. There is no appreciable change in the correlation time for 2 mM ^{14}N-labeled TEMPO solution in 85% glycerol. The rotational correlation time increases (~13 times) for 2 mM ^{14}N-labeled TEMPONE in the high-viscosity liquid mixture (pure water/glycerol). This ESR behavior implies that TEMPONE is a suitable spin probe for in vivo studies in high-viscosity biological fluids, which has the narrowest line width and also acts as a viscosity-prone spin. The DNP parameters are correlated with the ESR parameters. These results will be useful for the development of ESRI/OMRI modalities in high-viscosity fluids for in vivo imaging and also help in optimizing the ESR/OMR parameters in ESRI/OMRI techniques.

Finally, the dynamic nuclear polarisation studies were carried out for ^{15}N labeled carbamoyl-PROXYL in pure water and water/glycerol mixtures over a range of concentrations, viscosities, RF power level and ESR irradiation time. This study permits a clear understanding of the effect of molecular mobility upon Overhauser enhancement in high viscous liquid samples. The DNP factor started declining in the higher concentration region (~3 mM), which is mainly due to the ESR linewidth broadening. Therefore, the agent concentration is optimized as 2 mM for in vivo/in vitro ESRI/OMRI experiments. The change in the enhancement factor was also brought about by the water proton spin-lattice relaxation time. The increased DNP factor (35%)

was observed for solvent 2 compared with solvent 1. The observed copling parameter, ρ has low value for ^{15}N labeled carbamoyl-PROXYL in high viscous liquid samples (solvent 3 and solvent 4), which indicates that the weak coupling between the electron spin with the nuclear spin. The low value of coupling parameter, ρ leads to the reduction in the enhancement factor. The leakage factor, water proton spin-lattice relaxation time and longitudinal relaxivity were estimated. The viscosity was found to play an important role in the DNP mechanism. These results permit clear understanding of the factors determining enhancement and contrast in OMR images obtained by DNP.

CONCLUSIONS

The ESR spectroscopy studies on the reduction process of 1mM concentration of ^{14}N-labeled TEMPONE, TEMPO and TEMPOL in 1 mM concentration of ascorbic acid as a function of time, which reveals that the TEMPONE radical can act as a good redox sensitive spin probe for in vivo/in vitro ESR and OMR imaging techniques. The ESR studies on 2 mM concentration of ^{14}N-labeled deuterated MC-PROXYL and carboxy-PROXYL in liposomal solution as a function of liposome concentration using low frequency ESR spectrometers permit clear understanding of the permeable and impermeable nature of nitroxyl radicals. This study also illustrates that the ESR technique can be used to differentiate between the intra- and extra-membrane water by loading the liposomes vesicles with a lipid permeable nitroxyl spin probe. The ESR study of ^{14}N-labeled TEMPONE, TEMPO, carbamoyl-PROXYL and carboxy-PROXYL in pure water and pure water/glycerol mixture in the ratio of 15:85 illustrates that the molecular dynamics of nitroxyl radicals in high viscous medium such as blood, plasma, serum, plasma membrane and possibly in vivo. These results will be useful for the development of ESRI/OMRI modalities in high-viscosity fluids for in vivo

imaging and also help in optimizing the ESR/OMR parameters in ESRI/OMRI techniques. The dynamic nuclear polarisation studies of ^{15}N labeled carbamoyl-PROXYL in pure water and pure water/glycerol mixtures with different viscosities permits a clear understanding of the effect of molecular mobility upon Overhauser enhancement in high viscous liquid samples and molecular dynamics in biological fluids. It is envisaged that the results reported here may provide guidelines for the design of new viscosity prone nitroxyl radicals, suited to the biological applications of DNP.

These results will be useful for the designing and development of viscosity prone stable permeable and impermeable nitroxyl spin probes for ESR/OMR imaging techniques.