CHAPTER: 07

ACQUISITION PARAMETERS FOR HRMAS NMR OF DISEASED AND NORMAL TISSUES

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In previous chapter, we discussed about the physical aspects of data acquisition and discussed their effects. In this chapter, we will be discussing about the impact of acquisition parameters for HRMAS NMR spectroscopy in identification of disease biomarkers.

Temperature: Since lower temperature is necessary for the preservation of tissue metabolome, therefore it was suggested to perform experiments at a probe head temperature of $\leq 10^{o}C$. At this temperature, no significant variations were observed in metabolite concentrations in tissues for 60 minutes of acquisition time (Beckonert, Coen et al. 2010). At 3 ^{o}C, tissues are preserved in better conditions (Taylor, Wu et al. 2003; Jordan, He et al. 2007). The 303K temperature during acquisition was reported to affect triglycerides and TMAO resonance due to enzymatic degradation (Waters, Garrod et al. 2000).

Spinning rate: Not only temperature, spinning rate of rotor also has a profound effect on spectral resolution and tissue integrity. The HRMAS spectra acquired at slow spinning speeds like 600 Hz or 700 Hz is dominated by large water peak and its spinning sidebands, which appear at the frequency distances of integer multiples of the spinning speed of rotor. These spinning side bands could be minimized by two methods: increase in spinning speed and presaturation. The higher spinning speeds have higher centrifugal force which is capable of inducing mechanical stress in tissue. Such structural changes may affect spin-spin and spin- lattice relaxation times, diffusions coefficients of metabolites and related effect on line width and peak intensity. The spinning sideband suppression techniques like DANTE (delay Alternating with Nutations for Tailored Excitation), WATERGATE (Water suppression by Gradient Tailored Excitation), 1D-TOSS (TOtal Sideband Suppression) and 2D- PASS (Phase-Adjusted Spinning Sidebands) are available which can be applied at slow spinning rates for a better spectral acquisition and tissue preservation. Out of all these spectral editing sequences, DANTE combined with CPMG was more effectively reproducible and suitable for both sideband suppression as well as for quantification of small metabolites (Taylor, Wu et al. 2003). But higher rotor speeds are required for overcoming the anisotropic broadening as well as for pushing spinning side-bands outside the spectral region. Therefore, optimum rotor speed is necessary for not only improving the spectral resolution by minimising broadening and spinning artifacts but at the same time for maintaining the tissue integrity. 4-6 kHz of spinning rate has been found to be optimum for both criterias as these overcome the static resonance linewidth and maintain the tissue morphology. The spinning speeds differ with the tissue type, more water containing tissues with low structural integrity are less stable at higher spinning speeds (Beckonert, Coen et al. 2010). The 3 kHz speed cause some distortions at cellular level

but these do not hinder the histopathological analysis in prostate tissues. however, higher spinning rates than 3kHz cause significant structural changes in tissue biopsies (Taylor, Wu et al. 2003). Waybright et al. had also conducted HRMAS studies on differentiated and undifferentiated pre-adipocytes and observed that there was no microscopic evidence of cell lysis even after 2 hr of MAS at 3.5 kHz and 82% of these cells remain viable by trypan blue exclusion and only 15-19% of the larger, lipid-laden differentiated adipocytes were found to suffer some degree of cell lysis with MAS (Weybright, Millis et al. 1998). In a study on ischemic effect on brain biopsies, the authors have highlighted the perturbation in biochemical profile of tissues due to period of ischemia before freezing at -80^{o}C and its comparision with the mechanical stress induced by spinning. They had reported that the extended spinning at 4^{o}C leads to a significant increase in Asp, Cho, Cr, Gln, PCh and tCho due to mechanical tissue damage as compared to the metabolic changes occurring in ischemic brain. The significant increase in total creatine intensity was attributed to spinning induced mechanical injury in brain tissues (Opstad, Bell et al. 2008).