# Accurate Determination of Water-Macromolecule Exchange Independent of Reference Interaction

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**Introduction:** Water macromolecule exchange (WME) has been shown to play an important role in direct phase contrast imaging [1]. The WME effect induces an additional frequency separation between gray matter (GM) and white matter (WM) that complemented the susceptibility model [2], which was suggested by a number of studies [3-5] to be the dominant effect of phase contrast between GM and WM. Several factors contribute to the in vivo tissue susceptibility, including myelin, non-heme iron and macromolecules. It has been shown that the in vivo macromolecule diamagnetism can be on the same order of magnitude as that of iron [6]. In addition, a recent study [7] using bovine serum albumin (BSA) showed that the macromolecule WME is about half of the corresponding macromolecule susceptibility with opposite sign and therefore can be neglected in the overall phase susceptibility estimation. The study used dioxane instead of trimethyl-silyl-propionate (TMSP), which is commonly used as an internal NMR frequency reference and showed possible interaction with proteins [8]. On the other hand, dioxane has been shown to interact with water leading to shifts in resonance frequencies of water and dioxane [9] and therefore might also not be a reliable internal frequency reference for WME quantification. In this study, systematic studies were performed with both TMSP and dioxane to access their effect on the accurate determination of WME shift and susceptibility of macromolecules.

**Theory:** The phase contrast arises from two major effects: tissue susceptibility and WME. On the other hand, susceptibility induced frequency shifts depend on the tissue structure and geometry and are not additive directly to the WME shift, which is microscopic and does not have a geometric dependency. The total frequency shift then can be written as: $\Delta f_{total} = (\cos^2\theta - 1/3)\Delta f_{Susceptibility} + \Delta f_{Exchange}$, where $\theta$ is the angle between the local magnetization and the main field. Therefore, susceptibility and WME must be treated separately in a model that describes the demagnetization field.

**Method:** Two sets of samples were prepared for BSA-TMSP and BSA-Dioxane systems, with BSA concentrations of 0.36, 0.68 and 0.95 mM. A phosphate buffer with pH 7 was used. TMSP and 1,4-dioxane concentration in each system were varied from 0 to 100 mM. Pure BSA solutions were also measured with chloroform as external reference using a coaxial insert. Bruker 400 and 600 MHz spectrometer were used for frequency measurement. Acquisition bandwidths were 8.2K for 400 MHz and 9K for 600 MHz with 32 averages and a digital resolution of 0.13 Hz/Pt.

**Results and Discussion:** The WME shift was determined from the slope of the three BSA concentrations (Fig. 1). The value is 0.009 ppm/mM at 298K and is the same for different dioxane concentrations. On the other hand, this value changes dramatically for different TMSP concentrations, indicating a strong interaction between TMSP and BSA. For high TMSP concentrations, e.g. a TMSP-BSA ratio larger than 30, the WME shift maximized and stabilized over a larger concentration region (25 to 60 mM). The behavior is typical of a two-site chemical exchange that reaches steady-state equilibrium. Interestingly, the WME shift measured with TMSP at equilibrium is 0.025 ppm/mM and is almost the same as the BSA susceptibility reported in a previous study [7]. An explanation for this discrepancy is that dioxane interacts with water through a double hydrogen bond configuration [9] and shifts the water frequency to lower field, while TMSP does not interact with water (Fig. 2). Thus, the dioxane-water interaction creates a competition binding scenario and reduces the apparent WME shift. Our results suggested that the WME shift of BSA is almost the same as its susceptibility with opposite sign. This is further supported by measurements using chloroform as external reference (Fig. 3), where no concentration dependency was observed for different BSA concentrations.

**Conclusion:** Both TMSP and dioxane do not allow reliable separation of WME and susceptibility shifts. On the other hand, they can be accurately quantitated by a systematic measurement excluding the effects of reference interaction. The macromolecule WME shift is about the same compared to its susceptibility shift, with opposite sign. Therefore, WME effect should be included in models that describe tissue phase contrast.

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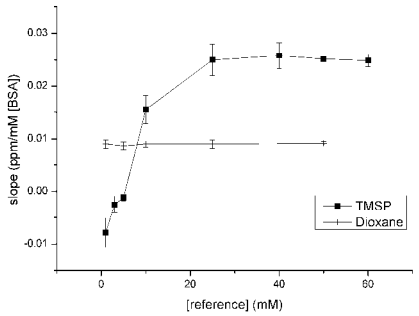


Fig 1: WME shift for different NMR references. TMSP changes with concentration and stabilize at high concentration (> 30 mM). Dioxane shift does not change in this concentration range.

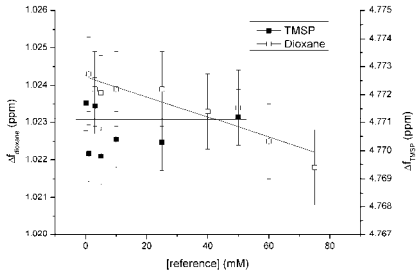


Fig 2: Exchange shifts between water and reference mixture. Dioxane showed strong concentration dependency while TMSP shift does not change.

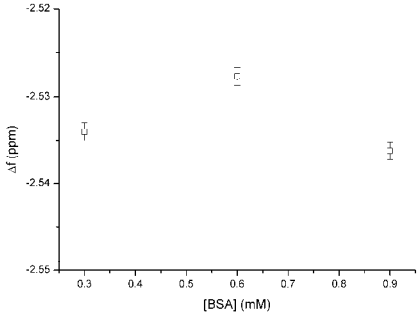


Fig 3: No concentration dependent frequency shift was observed for BSA with chloroform as external reference.

**References:** [1] K. Zhong *et al.* NeuroImage 40 (2008) [2] J. H. Duyn *et al.* PNAS 104 (2007) [3] A. Pfefferbaum *et al.* NeuroImage 47 (2009) [5] J. P. Marques *et al.* NeuroImage 46 (2009) [6] X. He *et al.* PNAS 106 (2009) [7] J. Luo *et al.* JMR 202 (1) (2010) [8] A. Shimizu *et al.* J. Biomol. NMR 4 (6) (1994) [9] K. Mizuno *et al.* J. Phys. Chem. B 107 (2003)