Fast-field cycling NMR is sensitive to the method of cross-linking in BSA gels

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Introduction

In contrast to conventional nuclear magnetic resonance (NMR) experiments where a static magnetic field is applied to a sample, the applied field in field-cycled NMR is altered during the experiment. Field-cycling allows measurements, for example of the spin-lattice relaxation rate (R_1), to be made as a function of the applied field. At several discrete field strengths the ^{1}H NMR and ^{14}N NQR frequencies coincide, allowing effective relaxation of the magnetisation from bulk water protons *via* the quadrupolar ^{14}N nucleus [**1**]. Therefore, in a plot of R_1 *versus* field, 'quadrupolar peaks' are often observed at these field strengths.

It has been suggested that quadrupolar peaks in proteinous samples result from interactions between sufficiently-bound nitrogenous functional groups and low-mobility water protons [**2**]. Furthermore, the amplitude of the quadrupolar peak has been shown to be proportional to protein concentration [**3**]. In this study, the quadrupolar peaks of gels of bovine serum albumin (BSA) formed by boiling or chemical cross-linking were examined.

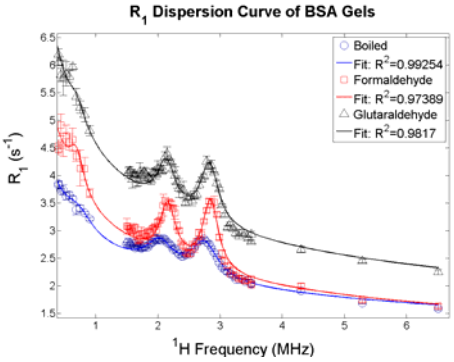

Figure 1: Dispersion curves of BSA gels generated by different cross-linking methods.

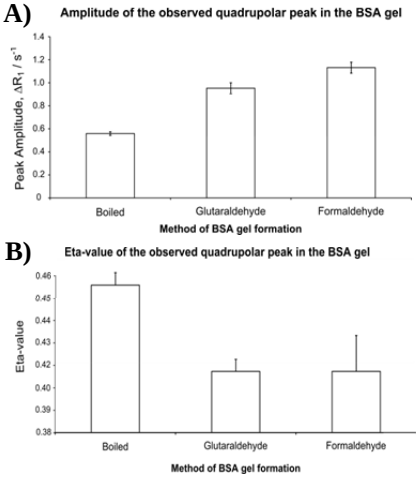

Figure 2: **A)** Amplitude and **B)** eta-value of the quadrupolar peak of cross-linked BSA gels.

Methods

BSA gels (final concentration 9% w/v) in 20.5 mM bicarbonate buffer (pH 7.3) were prepared by boiling or addition of formalin or glutaraldehyde (final concentration 12.5% w/v). Measurements of R_1 were made between 0.047–187.89 mT at 37 °C on a SMARtracer relaxometer (Stelar S.r.l., Mede, Italy). A power-law with Lorentzian-bell algorithm, derived from the literature [**3,4**], was fit to the data (Matlab 2012a, The Mathworks, Cambridge, UK; scripts developed by Lionel M. Broche, Aberdeen, UK).

Results

Dispersion curves were dependent on the method of gel formation (Figure 1). The quadrupolar peak amplitude was largest with formaldehyde (Figure 2A). The eta-value, describing the shape of the quadrupolar peaks, was similar with chemical cross-linking but larger if boiled (Figure 2B).

Discussion

Boiled BSA gels likely contain denatured protein in a network of fragments, monomers and higher aggregates [**5**] linked by various functional groups (e.g. amino, amide and sulphhydryl groups). Glutaraldehyde gels may contain BSA aggregates in a native configuration *via* the majority of free amino-groups [**6**]. Formaldehyde gels are likely cross-linked in a heterogeneous manner, involving fewer free amino-groups [**7,8**]. Therefore, quadrupolar peak amplitude and shape may be related to the macroscopic protein network, and/or the functional groups involved in gel cross-linking. These observations may be pertinent in the analysis of fixed tissue samples by field-cycled NMR, where any observed quadrupolar signals may be affected by the method of tissue fixation.

Acknowledgements

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References

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