

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

Brett W. C. Kennedy<sup>1</sup>, Lionel M. Broche<sup>1</sup>, G. Patrick Ashcroft<sup>2</sup> and David J. Lurie<sup>1</sup>

<sup>1</sup>Division of Applied Medicine, <sup>2</sup>School of Medicine and Dentistry, University of Aberdeen, UK

www.fjc-mri.org

## 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  $^1\text{H}$  NMR and  $^{14}\text{N}$  NQR frequencies coincide, allowing effective relaxation of the magnetisation from bulk water protons *via* the quadrupolar  $^{14}\text{N}$  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.

## 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

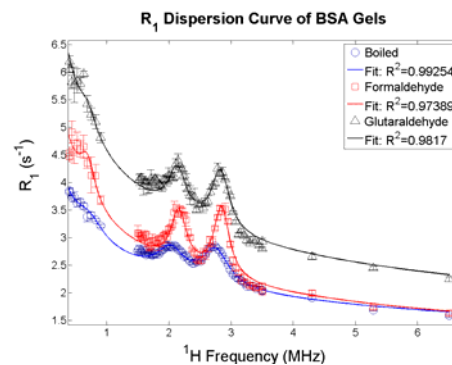
This work was supported by ARUK (grant number 19689).

Arthritis  
Research UK

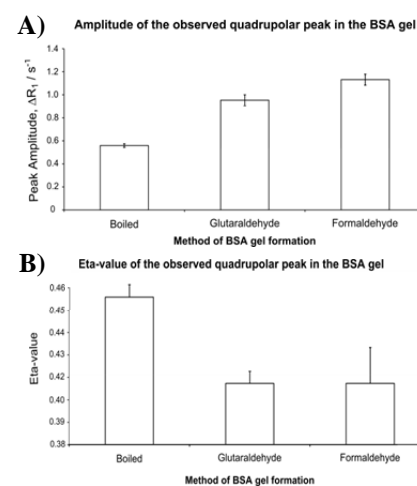
UNIVERSITY  
OF ABERDEEN

## References

[1] Winter, F. and Kimmich, R. (1982); 719(2), 292-298 [2] Sunde, E.P. and Halle, B. (2010); 203(2), 257-73 [3] Broche, L. M., Ismail, S. R., Booth, N.A. et al. (2012); 67(5), 1453-1457 [4] Winter, F. and Kimmich, R. (1982); 45, 33-49 [5] Aoki, K., Hiramatsu, K., Kimura, K., et al. (1969); 47(4), 274-282 [6] Hopwood, D. (1970); 24, 50-64 [7] Fraenkel-Conrat, H. and Mecham, D. K. (1949); 177, 477-486 [8] Fraenkel-Conrat, H. and Olcott, H. S. (1948); 70(8), 2673-2684



**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.