1D NMR methods for analysis of cross-linking properties in hyaluronan hydrogels

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Hydrogels consist of three-dimensional cross-linked polymer networks, their physical properties can vary a lot depending on the characteristics of the structure. Parameters which describe these networks are therefore important to understand the differences in properties as well as to facilitate the design of hydrogels with desired properties. One important parameter that describe such a network is the degree of cross-linking which combines the degree of modification and the effective cross-linker ratio.[1] Another parameter that might influence the physical properties of the hydrogel is the substitution position of the cross-linker on the polysaccharide. Using NMR spectroscopy and LC-MS/MS, we determined the substitution pattern in hyaluronic acid (HA) hydrogels cross-linked with 1,4-butanediol diglycidyl ether (BDDE). HA-BDDE hydrogels are highly viscous so to enable the analysis the hydrogel was first enzymatically degraded and the digest was subsequently separated into size dependent fractions by preparative HPLC. Structural analysis of the fragments demonstrated that all four hydroxyl groups of the HA repeating disaccharide could be substituted by BDDE.[2] Further we developed 1D NMR methods for analysis of the relative amount of substitution at the four positions and for the degree of cross-linking. Both parameters can be assessed from the same simple sample preparation which requires no chromatographic separation.[3]

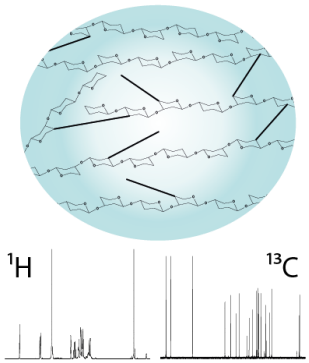

Figure 1. 1D NMR methods provides unique information on the structure of hydrogels

[1] L. Kenne et al. *Carbohydr. Polym.* **2013**, *91*, 410-418
[2] F. J. Wende et al. *Carbohydr. Polym.* **2016**, *136*, 1348-1357
[3] F. J. Wende et al. *Carbohydr. Polym.* **2017**, *157*, 1525-1530