

## Calibration of the diffusion coefficients of the FCS standard Rhodamine 6G (Rh6G) in aqueous solutions

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Precise diffusion measurements of Rhodamine 6G (Rh6G) dissolved in D<sub>2</sub>O at concentrations between 1 and 410 mM were carried out in the temperature range from 280 to 320 K using pulsed field gradient nuclear magnetic resonance (PFG-NMR) [1, 2]. A spin-echo NMR sequence with solvent suppression (PGSE-WATERGATE) [3] was applied to measure the diffusion coefficients of Rh6G in low-concentration ( $C_{\text{Rh6G}} = 1, 4.5, \text{ and } 25 \mu\text{M}$ ) aqueous solutions. The precise determination of the diffusion coefficients in this low-concentration range was made possible by using a cryogenically cooled NMR probe and by the effective solvent suppression of the PGSE-WATERGATE sequence. The temperature and concentration dependent diffusion coefficients of Rh6G can be used as calibration references in fluorescence correlation spectroscopy (FCS). The diffusivity of Rh6G decreases with increasing Rh6G concentration, which can be explained by dimerization of Rh6G molecules. Besides measuring the diffusivity of Rh6G, the diffusion coefficient of the solvent in the same system could be determined in parallel. The temperature dependence of Rh6G diffusion ( $H_a = 0.23 \text{ eV}$ ) is stronger than that of water diffusion ( $H_a = 0.20 \text{ eV}$ ) in the same solution. The isotope effect of the solvent on the diffusion coefficient of Rh6G is determined by FCS measurements on Rh6G dissolved in both D<sub>2</sub>O and H<sub>2</sub>O.

### References

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