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# Estimating microstructural length scales in κ-carrageenan hydrogels by PFG NMR nanoprobe diffusometry

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We use PFG NMR to measure hindered self-diffusion of spherical, monodisperse diffusional nanoprobes in a polysaccharide network. These nanoprobes have different diameters in the 1–10 nm range, but identical inert (PEG) surfaces. We use Johnson's model of particle self-diffusion in fibrous networks to estimate the polymer strand thickness and to obtain a measure for the network mesh size. Johnson's model takes into account the obstruction effect, and the degree of obstruction is independent from the arrangement of the polymer strands at low polymer concentrations. Probe particles with different sizes provide consistent predictions of strand thickness in this concentration regime. This allows us to infer a structural length scale close to the average mesh size of the polymer network.

## Introduction

Biopolymer hydrogels consist of a water phase that is expanded by a percolating polymer network, giving rise to a porous and tortuous microstructure. If biopolymer hydrogels were considered to be porous media, we could attempt to use established pulsed field gradient (PFG) NMR methodology to characterize their microstructure by studying the self-diffusion of water within the pores. PFG NMR is widely used to study structural properties of, and transport properties in foods and porous media in general.[1] PFG NMR probes the ensemble average propagator $\bar{P}(r,t)$ that describes the probability of a particle moving over $r$ during observation time $t$. For the well-known case of Brownian motion in an isotropic homogeneous medium, the propagator is a Gaussian function,

$$\bar{P}(r,t) = (4\pi Dt)^{-3/2} e^{-\frac{r^2}{4Dt}},$$

whose width is proportional to the diffusion coefficient $D$, a scalar property in an isotropic medium. In a porous medium, the diffusion of small molecules may be restricted within the pores, leading to the shape of the propagator changing as a function of time, providing structural information about amongst others pore size. However, due to the very open network structure of hydrogels, established methods that infer microstructural information from PFG NMR measurements of small molecules (such as water) moving within in porous media generally do not provide the desired structural information.

Alternatively, we can use larger "diffusional nanoprobes" to obtain quantitative microstructural parameters, complementing information obtained through microscopy.[2] The tortuous nature of the networks with respect to particles with sizes on the order of the network mesh size leads to their self-diffusion coefficients being significantly lower than in bulk water. Particles larger than the structural features of the polymer network are completely immobilized.[2-4] The latter effect has been observed in polysaccharide hydrogels if the size of the particles exceeds a certain threshold, which likely corresponds to the size of the smallest interstitials between the polymer strands.[5] For the largest part, however, diffusion of nanoparticles remains hindered but unrestricted. The pertinent question is what information can be obtained from this non-restricted but hindered diffusion of nanoparticles. Physical models of particle self-diffusion in hydrogels have been used to relate the reduction of self-diffusion coefficients to polymer concentration, polymer strand thickness, nanoparticle size and network mesh size.[2,6] Many of these models try to capture what is colloquially known as the "obstruction effect", i.e. the effect that the rigid and tortuous polymer network imposes an increased path length for particles moving between two points in the network. Such obstruction models predict long-term, average particle diffusion coefficients (assuming simple Brownian motion and a static network structure).

In this paper we explore whether physical models of diffusion can adequately describe nanoparticles moving unrestricted through a polymer gel by using a set of equivalent spherical nanoparticles with identical surface chemistry. We will compare the structural descriptors obtained from these chemically equivalent, but differently sized particles. As a model biopolymer we will use κ-carrageenan, a naturally sourced linear polysaccharide that is widely used industrially as a gelling agent. Gelation of κ-carrageenan occurs upon cooling a warm aqueous solution, during which the polymer coils first form helices that subsequently aggregate in a side-by-side manner.[7,8] The coil-to-helix transition, which is essential for eventual gelation, is very sensitive for binding of cations such as potassium, calcium, or sodium ions, strongly influencing the microstructure and elastic strength of the gels[9]. The microstructure of κ-carrageenan gels can therefore essentially be controlled by the choice of cations, making it an elegant model system for microstructural studies.

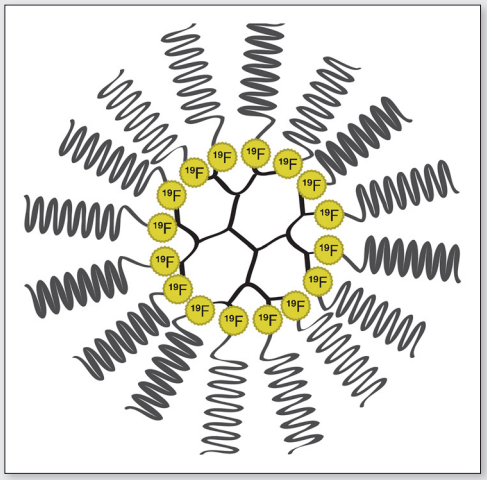


Figure 1. Design of diffusional nanoprobes with dendritic cores (black) of different generations, and inert polyethylene glycol (PEG) coronas (grey). Fluorine labels (circles) are situated in the interior of the particles, underneath the PEG corona. An elaborate description of this design is found in ref 5.

## Materials and methods

### Sample preparation

κ-Carrageenan gels were prepared by suspending κ-carrageenan powder (2 wt%) in a solution of sodium chloride (200 mM), potassium chloride (20 mM), and labelled dendritic nanoparticles in water (0.1 wt%), followed by stirring and heating to 80 °C for 15 minutes. This allows κ-carrageenan to dissolve. The solutions are subsequently allowed to cool down to room temperature during which gelation takes place. Before cooling, we mix the polymer solution with a dilute solution of dendritic nanoparticles. These dendritic nanoparticles are $^{19}$F-labelled, PEGylated (and hence nonsticky), and have generation-1, -3 and -5 (G1, G3, G5) poly(propylene imine) (PPI) cores as presented elsewhere[5] (SyMO-Chem B.V., Eindhoven, Netherlands). These nanoparticles have hydrodynamic diameters between 2.8 and 6.9 nm. A schematic of their structure is shown in Figure 1. Besides these particles also 4-amino-2,2,6,6-tetramethylpiperidine (ATEMP) was used as a diffusional probe.

### PFG NMR diffusometry

Diffusion-ordered spectroscopy (DOSY) experiments were performed with a Bruker Avance II spectrometer at 300 MHz resonance frequency for $^{1}$H, equipped with a Bruker diff25 gradient probe (maximum pulsed field gradient (PFG) intensity 9.60 T/m). The probe was equipped with a 10-mm RF insert that could be tuned to the $^{1}$H and $^{19}$F NMR resonance frequencies. Experiments and data analysis were performed as outlined previously[3]. In short, DOSY experiments based on a spin echo were performed by stepwise variation of the gradient pulse amplitude at an effective gradient pulse duration of 5 ms, whilst keeping the diffusion-observation time at 200 ms. For $^{1}$H NMR measurements, the minimum gradient amplitude was chosen to be high enough to attenuate the $^{1}$H NMR signal of water almost completely. The attenuation of the $^{1}$H PFG NMR echo intensity of the PEG corona as a function of increasing gradient amplitude is described by a sum of Stejskal-Tanner-type exponentials $I/I_0 = Ae^{-bD}$ and $b = (\gamma\delta g)^2(\Delta - \delta/3)$, where $I/I_0$ is the signal attenuation, $D$ the diffusion coefficient, $\gamma$ the gyromagnetic ratio, $\delta$ the width of the gradient pulse, $g$ the gradient amplitude and $\Delta$ the diffusion-observation time.[10] In principle, $^{1}$H and $^{19}$F NMR can be used interchangeably, where $^{19}$F NMR offers higher selectivity at the cost of sensitivity. For G3 and G5 particles, $^{1}$H NMR was used to allow more sensitive filtering of an immobilized particle fraction. For G1 particles, such a fraction was absent so that $^{19}$F NMR was more convenient.

## Results and discussion

### Effect of obstruction and solvent viscosity

The self-diffusion coefficients of a set of dendritic particles, a low-molecular weight probe (ATEMP) and water in gels prepared with a range of κ-carrageenan concentrations have been presented in Figure 2. For all species the self-diffusion coefficients decrease with κ-carrageenan concentration. Note that the effect of κ-carrageenan concentration is most pronounced for the dendritic particles. This strong concentration effect is not a surprising observation and has been described and modelled many times for nanoparticles in various polymeric solutions and gels.[6] The availability of monodisperse spherical dendritic nanoparticles with inert surfaces provides a unique opportunity to assess consistency of obstruction model

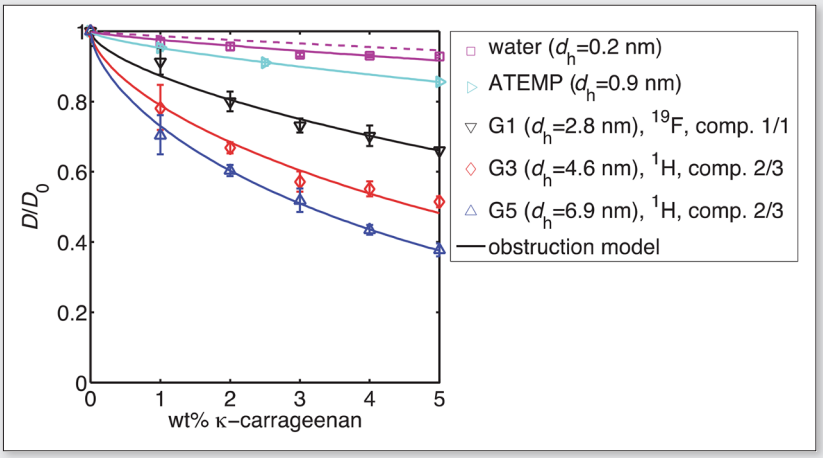


Figure 2. PFG NMR diffusivities of dendritic nanoparticles, ATEMP (4-amino-2,2,6,6-tetramethylpiperidine), and water in κ-carrageenan gels at 20 mM K+/200 mM Na+. Straight lines represent model fits that account for polymer network obstruction and water viscosity due to presence of sucrose (see main text). The dashed line represents model prediction based on average polymer strand thickness (3.0 nm) observed for ATEMP and dendritic nanoparticles PFG NMR data of dendritic particles.

predictions throughout a considerable nm-scale particle size range. We first considered whether –besides obstruction– also other effects could have an effect on self-diffusion behaviour of dendritic nanoparticles. A hitherto neglected effect is increased local viscosity of the aqueous solvent. κ-Carrageenan is known to contain low amounts of sugars, and indeed the (static) $^1$H NMR spectra of κ-carrageenan gels show the presence of freely dissolved sucrose. The concentration of sucrose in each gel was quantified by integration of the corresponding $^1$H NMR signal and comparison with a reference solution. Thus, self-diffusion coefficients of the particles, ATEMP and water were corrected for the increased viscosity due to the sucrose[11] by using the Stokes-Einstein relation, which relates solution viscosity to the diffusion coefficients of spherical particles in those solutions. It is the corrected diffusion coefficients that are presented in Figure 2.

For water, besides enhanced water viscosity due to sucrose, also the effect of fast proton exchange with the stationary carrageenan matrix was accounted for. Fast exchange would influence the apparent diffusion coefficient as

$$D_{app} = D_S f_S + D_M f_M$$

where $f_S$ and $f_M$ are the fractions of stationary (carrageenan-bound) and mobile (water-bound) mutually exchangeable protons, respectively. If we assume that $D_S = 0\,m^2/s$, we simply have to calculate the molar fraction of exchangeable protons (i.e. hydroxyl protons) on the carrageenan chain relative to the number of water protons. This exercise leads to the conclusion that fast proton exchange lowers the apparent water diffusion coefficients by not more than 1% (depending on carrageenan concentration) for which the data in Figure 2 have been corrected.

## Quantification of network features using an obstruction model

The corrected self-diffusion data were fitted to the diffusion-obstruction model of Johnson et al.[12], which is based on the notion that the degree of hindrance of probe diffusion in a polymer network is the product of hydrodynamic (*F*) and steric (*S*) contributions:

$$\frac{D}{D_0} = F\left(\frac{r_s}{\sqrt{\kappa}}\right) S(\alpha),$$

where $r_s$ is the particle radius, κ the Darcy permeability and α the "effective" volume fraction of the polymers adjusted for the particle size (as particles are excluded from the volume of solvent surrounding the polymer strands). In their paper, Johnson et al. showed that for many polymer networks this equation can be written as:

$$\frac{D}{D_0} = e^{-0.84\alpha^{1.09}}\left(1+\left(\frac{r_s^2}{\kappa}\right)^{1/2}+\frac{1}{3}\frac{r_s^2}{\kappa}\right)^{-1} \quad \text{with} \quad \alpha = \varphi\left(\frac{r_s+r_f}{r_f}\right)^2$$

$$\text{and } \kappa = 0.31 r_f^2 \varphi^{-1.17},$$

where $r_s$ is the nanoparticle radius and φ the polymer volume fraction. Using the density of carrageenan to recalculate polymer weight fraction into volume fraction, we can use this expression to fit the polymer strand radius $r_f$ from our data. Figure 3 shows a schematic depiction of a gel network, with an average polymer strand radius $r_f$ and mesh size $d_m$. The effective strand thickness probed by the nanoparticles in the κ-carrageenan gels will ultimately depend on the size of the particles, as they can only probe structural details larger than their own size. Depending on the conformation of the κ-carrageenan chains, they will therefore probe the effective thickness of the fibres in that particular conformation (e.g., the thickness of helices or aggregated helices, but not the thickness of the molecular backbone). The solid lines in Figure 2 are least-squares fits to the obstruction model. The particle sizes that were put into the model, and the polymer strand radii that result from this fit can be found in Table 1. Despite of the probe particle sizes $r_s$ ranging across almost an order of magnitude, the obstruction model predicts a strand thickness of 3.0 ± 0.2 nm from the self-diffusion behaviour of the dendritic particles and ATEMP.

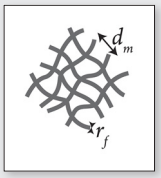


**Figure 3.** Schematic depiction of a polymer network with strand radius $r_f$ and "mesh size" $d_m$.

It turns out that if the effective polymer volume fraction $\alpha < 0.5$, as in all the experiments in this work because of the low polymer concentrations used, the way in which the polymer strands are oriented (e.g. randomly or in an orthogonal array) does not significantly influence the obstruction factor *S*.[12] For a hypothetical cubic-orthogonal array of polymer strands, we can use the dimensions of the cubic repeating unit as a measure for the "mesh size" $d_m$ of the network. The volume of three orthogonal cylinders with radius $r_f$ in a cubic repeating unit with edges *d* equals $3\pi r_f^2 d_m$. The volume fraction of polymer per repeating unit equals φ, whilst the volume of each repeating unit is $d_m^3$. This means that $\varphi = 3\pi r_f^2 d_m / d_m^3$, so that $d_m = r_f\sqrt{(3\pi/\varphi)}$. The size of the repeating unit $d_m$ merely provides a length scale for the mesh size, which is an ill-defined term in an array of randomly oriented polymer strands. For the carrageenan gels, with an average polymer strand thickness of 3.0 nm, length scale *d* ranges from 0.05 µm for the 1 wt% gel down to 0.02 µm for the 5 wt% gel (Figure 4). One can observe that the nanoparticle size range fall in the same order of magnitude as length scale $d_m$.

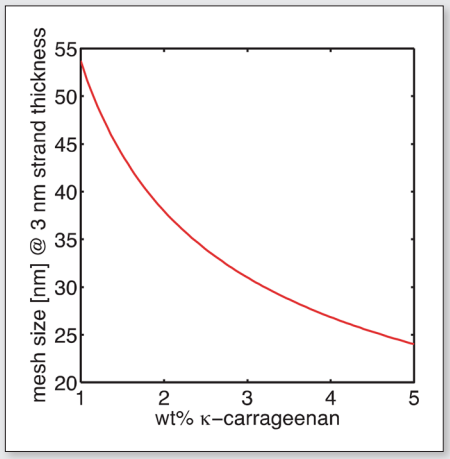


**Figure 4.** Size of the repeating unit $d_m$ ("mesh size") of an array of orthogonal polymer strands with a diameter 3.0 nm as a function of κ-carrageenan-concentration.

Table 1. Polymer strand diameters apparent from fits of PFG NMR data of different particles in κ-carrageenan gels with an obstruction model.

| Particle name | Hydrodynamic particle diameter ($2r_s$) | Apparent polymer strand diameters ($2r_f$), from Johnson's model (ref 12) |
|---|---|---|
| $H_2O$ | ~0.2 nm | 1.3 nm |
| ATEMP | ~0.9 nm | 2.8 nm |
| G1 dendrimer | 2.8 nm | 2.9 nm |
| G3 dendrimer | 4.6 nm | 2.8 nm |
| G5 dendrimer | 6.9 nm | 3.2 nm |

### Indications for local variations in water diffusivity

The polymer strand thickness derived by modelling the obstruction of the self-diffusion of water (Table 1) is much smaller (1 nm) than the values obtained from the dendritic nanoparticles and ATEMP (3.0 nm). This means that there must be an additional phenomenon that lowers the water self-diffusion (on top of obstruction and increase in water viscosity due to presence of sucrose), leading to this underestimation of the strand thickness. In Figure 2 the dashed line represents the prediction of the self-diffusion behaviour of water from the strand thickness derived from modelling the behaviour of the dendritic particles and ATEMP. The small but consistent difference between experimental water self-diffusion constants and predictions from the obstruction model indicates that there is a yet unaccounted effect. Possibly, the deviations are caused by the presence of polymer hydration layers in which water mobility is lower than in the bulk. The larger particles that cannot approach the polymer strands closely due to size exclusion effects would not probe the higher water viscosity in the hydration layers. Local measurements of water viscosity are needed to confirm this suspicion as PFG NMR will only provide the ensemble average diffusivity.

## Conclusion

We have tested whether the self-diffusion behaviour of water, a low-molecular weight probe (ATEMP) and nm-scale dendritic nanoparticles in biopolymer gels can be modelled in a consistent manner. The self-diffusion constants were corrected for increased viscosity due to presence of sucrose, and in the case of water also for proton exchange. For the dendritic particles and ATEMP their (corrected) self-diffusion behaviour consistently predicted a polymer strand thickness of ~3.0 nm. The (corrected) self-diffusion behaviour of water could however not be fully explained by presence of sucrose, proton exchange and network obstruction. This deviation might lie in the presence with regions of higher-viscosity surface water that are inaccessible for the larger particles. For the dilute polymer gels studied here safe estimates can be made of the average mesh size of the network. Such information can aid in the validation of microscopic images of biopolymer networks.

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