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Review

Nanoparticle diffusometry for quantitative assessment of submicron structure in food biopolymer networks: A review

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At the submicron scale, food biopolymer networks can be visualized by a wide array of microscopic techniques, but these methods are mostly invasive and require careful image analysis in order to quantify network features. 'Nanoparticle diffusometry' provides a non-invasive alternative to infer quantitative submicron structural information about biopolymer networks. In this approach, spectroscopy-based methods are used to monitor hindered diffusion of nanoparticles due to network obstructions. Both rigid-spherical and flexible nanoparticles can be used and models are available to derive structural network parameters. A range of applications to polysaccharide and protein sols and gels has been described. The approach offers opportunities to assess (sub-) micron scale network heterogeneity and changes in submicron structure under dynamical conditions such as shear or aging.

Introduction

In foods, the structural characteristics of micro- and mesoporous biopolymer networks underlie critical macroscopic product functionalities, such as taste, mouthfeel, controlled release and delivery characteristics, and water retention capacity and barrier properties. The key to understanding these macroscopic functionalities lies in understanding how biopolymer strands and globules form networks by aggregation, crosslinking and/or entanglement (Lorén, Nydén, & Hermansson, 2009; Ross-Murphy, 1995; van der Sman & van der Goot, 2009). Analytical methods that can be used to characterize biopolymer network sub-micron structures can help us to unravel these complicated structure-function relationships. From the food manufacturing perspective, it is important to design and control structures at short length scales and here such methods are indispensable to predict product functionality. Although direct visualization of biopolymer networks can be provided by electron microscopy techniques, these methods suffer from several shortcomings. Sample preparation methods are invasive and likely to influence network properties. Moreover, careful image analysis is necessary in order to quantify structural descriptors and dynamic events in time cannot be studied on the same sample. Imaging techniques that provide nanometer resolution generally cover only a small field of view, which compromises representative assessment of heterogenous systems.

Over the last years, we have witnessed the advent of 'nanoparticle diffusometry', an analytical method in which nanoparticles are used as "probes" that move through the biopolymer matrix by molecular self-diffusion (Brownian motion). Nanoparticles have diameters that are smaller, but of the same order of magnitude as the mesh size of the biopolymer network. Therefore, self-diffusion of

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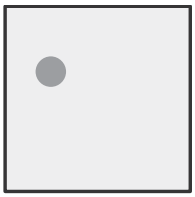
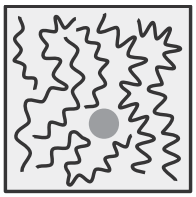
nanoparticles through the network is hindered by the presence of the biopolymer fibers or chains, but the nanoparticles are not immobilized, as larger (micron-size) particles would be. Hindered self-diffusive behavior of nanoparticles can be used to quantitatively infer submicron structural descriptors, such as mesh size or polymer strand thickness, by using models that describe nanoparticle self-diffusion in obstructing networks. Only nanoparticles that have no specific chemical interactions with the matrix are used, and nanoparticles are dispersed or dissolved at the lowest possible concentration at which their self-diffusion can be experimentally detected. Self-diffusional behavior of small organic molecules or water molecules is generally not significantly affected by biopolymer networks, which are very open compared to the sizes of such small molecules (Gottwald, Creamer, Hubbard, & Callaghan, 2005; Mariette, Topgaard, Jönsson, & Söderman, 2002). Besides this, these small molecules often engage in specific physico-chemical interactions with the network, which complicates interpretation of experimental diffusometric data. Experimental methods that have been used to monitor the self-diffusion of nanoparticles in biopolymer networks are fluorescence and optical microscopy, single particle tracking, and nuclear magnetic resonance (NMR). Nanoparticle diffusometry should be distinguished from direct assessment of restriction casu quo barrier functionality of a biopolymer material towards specific (bio)macromolecules. Using this approach, barrier properties of foods and other natural substances such as fish meat (Carvajal-Rondanelli & Lanier, 2010), mucus and mucin (Lafitte, Söderman, Thuresson, & Davies, 2007; Lafitte, Thuresson, Jarwoll, & Nydén, 2007; Olmsted *et al.*, 2001; B. S. Schuster, Suk, Woodworth, & Hanes, 2013) towards specific (bio)macromolecules have been described. The

Table 1. Three cases for nanoparticle diffusion: (A) Simple viscous liquids, (B) Sols of flexible and semiflexible (bio)polymers, (C) Rigid/fibrous networks, such as gels.

	A. Simple, viscous liquids	**B. Sol(ution)s of flexible and semiflexible polymers**	**C. Rigid/Fibrous networks (gels)**
			
van Hove self-correlation function	Gaussian	Non-Gaussian behavior has only been observed in semiflexible polymer solutions for nanoparticles with a size comparable to the structural length scale. (Valentine *et al.*, 2001)	(1) Non-Gaussian at the time scale at which the nanoparticles' diffusion path length is close to the structural length scale (Valentine *et al.*, 2001) (2) Gaussian at long time scales (central limit theorem), if nanoparticles are not permanently constrained and the system is not completely fractal (Lorén, Nydén, *et al.*, 2009; Netz & Dorfmüller, 1995; B. Wang, Kuo, Bae, & Granick, 2012)
Information content	*Liquid homogeneity*	*Network homogeneity in case a Gaussian distribution is observed*	(1) *Network heterogeneity* (2) *Tortuosity*
Time-dependency of mean-square displacement	Linear (Fickian)	(1) Fickian in case particles are smaller than the structural length scale (Cai, Panyukov, & Rubinstein, 2011) (2) Non-linear (anomalous) in case nanoparticle size is comparable to or larger than the structural length scale (Cai *et al.*, 2011; Wong *et al.*, 2004)	Anomalous at the time scale at which nanoparticles become constrained (Valentine *et al.*, 2001)
Information content	*Solvent viscosity*	(1) *Solvent viscosity* (2) *Local network elasticity (microrheology)*	*Network heterogeneity, pore size*

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approach works very well in the design of products that are effective barriers against those specific (bio)molecules, where measurement of self-diffusion coefficients is a direct measure of barrier functionality, unlike microscopic images, which only provide an indirect assessment. These studies, however, do not aim at quantitatively deriving the underlying structural network descriptors through physical models of diffusion of nanoparticles that are designed not to have specific interactions with the network. Also different from nanoparticle diffusometry is microrheology, where micron-size colloidal particles are used to locally probe elastic properties of the network. Micron-size particles locally probe *macroscopic* material properties because their size is much larger than mesoscopic structural features. Therefore, the particles diffuse in an effectively homogenous medium. In microrheology, the time-dependent mean square particle displacement $\langle r^2(t)\rangle$ due to diffusion is recorded to derive the frequency-dependent complex visco-elastic modulus (G', G'') via the generalized Stokes–Einstein equation (Mason & Weitz, 1995). Microrheology applications in food gels and emulsions mostly rely on particle tracking and diffusing wave spectroscopy (Moschakis, 2013).

Nanoparticle diffusometry has already been used to characterize the microstructures of polysaccharide and protein networks. Examples include kappa-carrageenan, galactomannan, alginate, whey and casein systems. For these systems, parameters such as polymer strand radii, mesh sizes and protein aggregate voluminosities were derived (for references: Table 3). Here, we will review a range of nanoparticle diffusometry applications in food science. We will also review relevant models, instrumental methods and physico-chemical properties of various nanoparticles. We will extract principles and examples from literature directly related to foods. Yet unexploited opportunities for foods will be illustrated by recent examples from adjacent fields, which also rely on structural characterization of (bio)polymer networks. We will finalize with perspectives and trends in nanoparticle diffusometry.

Models for nanoparticle diffusion in biopolymer networks

As opposed to the use of diffusometry to assess barrier functionality towards specific (bio)molecules, quantitative nanoparticle diffusometry is a model-based approach to material characterization, aimed at obtaining quantitative network parameters. Models for particle diffusion in polymer sols and gels build on established relationships describing Brownian motion in simple liquids, where the diffusion coefficient follows from the Einstein–Smoluchowski relation $Df = k_bT$, where D is the self-diffusion coefficient ($m^2\ s^{-1}$), f is the drag coefficient (kg s^{-1}), k_b is the Boltzmann constant and T is temperature (K). For spherical particles in a simple liquid, the magnitude of the drag coefficient f is given by Stokes' law $f = 6\pi\eta r$, where η is the dynamic viscosity (Pa s) and r is the hydrodynamic particle radius (m). Combining these equations gives the Stokes–Einstein relationship $D = k_bT/6\pi\eta r$ for the diffusion coefficient of spherical particles in simple liquids. In this case the nanoparticle displacement probability distribution

Table 2. Models for diffusion of spherical nanoparticles in rigid polymer gels that can be used to derive quantitative network descriptors. All models assume non-sticky nanoparticles. Meaning of the symbols: r_s is the solute (particle) radius, r_f is the polymer fiber radius and φ is the polymer volume fraction. The model by Cukier *et al.* (Cukier, 1984) has not been listed since it has been shown to be valid mainly for small diffusants in semidilute polymer solutions, but to break down for larger diffusants (Masaro & Zhu, 1999). The model by Ogston *et al.* (Ogston, 1958) has been criticized for lack of agreement to experimental data, which mainly show a Gaussian dependence of diffusivity on particle radius: $\frac{D}{D_0} \propto e^{-r_s^2}$ (Amsden, 1998b). Adapted with permission from Amsden (1998b). Copyright 1998, American Chemical Society.

Network properties	Model	Class	References
Uniform random suspension of fibers	$\frac{D}{D_0} = e^{-\frac{r_s+r_f}{r_f}\sqrt{\varphi}}$	obstruction effect	(Ogston, 1958)
Linear, rigid polymer gel	$\frac{D}{D_0} = \left(1+\frac{2}{3}\alpha\right)^{-1}$ with $\alpha = \varphi\left(\frac{r_s+r_f}{r_f}\right)^2$	obstruction effect	(Tsai & Strieder, 1986)
Closely-packed hard spheres	$\frac{D}{D_0} = \left[1-\frac{\varphi}{0.571}\right]\left[1+\varphi^2(1.459-11.04\varphi^2\right]$	obstruction effect	(Speedy, 1987)
Linear, rigid polymer gel	$\frac{D}{D_0} = e^{-0.84\alpha^{1.09}}$ with $\alpha = \varphi\left(\frac{r_s+r_f}{r_f}\right)^2$	obstruction effect	(Johansson, Skantze, & Loefroth, 1991)
Linear polymer gel with very stiff chains, $r_s > 2$ nm	$\frac{D}{D_0} = \frac{e^{-0.84\alpha^{1.09}}}{1+\left(\frac{r_s^2}{k}\right)^{\frac{1}{2}}+\frac{1}{3}\frac{r_s^2}{k}}$ with $\alpha = \varphi\left(\frac{r_s+r_f}{r_f}\right)^2$ and $k = 0.31r_f^2\varphi^{-1.17}$	obstruction effect and hydrodynamics	(Johnson, Berk, Jain, & Deen, 1996)
Linear polymer gel with very stiff chains, $\varphi < 0.40$, $r_s > 2$ nm	$\frac{D}{D_0} = \left(1+\frac{2}{3}\alpha\right)^{-1}e^{-\pi\varphi^{0.174\ \ln\left(51.6\frac{r_f}{r_s}\right)}}$ with $\alpha = \varphi\left(\frac{r_s+r_f}{r_f}\right)^2$	obstruction effect and hydrodynamics	(Clague & Phillips, 1996)
Linear, rigid polymer gel	$\frac{D}{D_0} = e^{-\pi\left[\frac{r_s+r_f}{k_s\sqrt{\varphi}+r_f}\right]^2}$ with k_s an adjustable parameters depending on the flexibility of the polymer chains.	obstruction effect and scaling law	(Amsden, 1998a)

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Table 3. Overview of instrumental methods relevant for nanoparticle diffusometry. Indicated are the time and length scales of self-diffusion that are covered by the various methods, the demands that methods impose on nanoparticles and strength and weaknesses of the various methods.

Method (refs.)	Time and length scales	Nanoparticle requirements	Strengths and weaknesses
Fluorescence correlation spectroscopy (FCS) (Rathgeber, Beauvisage, Chevreau, Willenbacher, & Oelschlaeger, 2009)	**Time scale** lower: 10^{-3} ms upper: up to 10^{4} ms **length scale** 10^{0} μm	– Fluorescent – Size < 10^{2} nm	+High time resolution +Ensemble information +Spatially resolved –Optical transparency needed
Fluorescence recovery after photobleaching (FRAP) (Axelrod, Koppel, Schlessinger, Elson, & Webb, 1976; Braeckmans, Peeters, Sanders, De Smedt, & DeMeester, 2003)	**Time scale** time scales cannot be directly probed **length scale** 10^{1}–10^{3} μm	– Fluorescent	+Stationary fraction can be quantified +Spatially resolved –Only one single, long-term diffusion coefficient can be determined
Pulsed-field gradient nuclear magnetic resonance spectroscopy (PFG NMR) (Callaghan, 2011)	**Time scale** 10^{0}–10^{3} ms **length scale** 10^{1}–10^{6} μm (diffusion-MRI)	– Contains NMR-active nuclei (such as ^{1}H) with liquidlike reorientation dynamics	+Ensemble information +No optical transparency needed +Propagator and diffusion coefficient +Spatially resolved +Signal separation of diffusing and static compounds (DOSY, DRCOSY) –Low sensitivity
Single particle tracking (SPT) (Braeckmans *et al.*, 2010; Filipe, Hawe, & Jiskoot, 2010)	**Time scale** lower: 1 ms upper: field-of-view **length scale** 10^{1}–10^{3} μm	– High diffraction index, or fluorescent – No specific size limit	+Information about individual particles retained +Spatially resolved –Many observations required for adequate statistics -Possible depletion effects near cover glass surface

(also van Hove self-correlation function or ensemble-average propagator) is Gaussian at all time scales and the mean-square particle displacement increases linearly with time ("Fickian" diffusion) (Table 1, case A).

For simple liquids the drag coefficient is determined by hydrodynamic interactions of the particle with solvent and solutes. For the more complex case of self-diffusion in sols of flexible and semiflexible polymers (Table 1, case B), the van Hove self-correlation function is Gaussian if the movement of the polymer chains is fast with respect to particle mobility. A non-Gaussian van Hove self-correlation function has only been observed in solutions of *semi*flexible F-actin filaments at the millisecond time scale (Valentine *et al.*, 2001), suggesting that observation of a non-Gaussian van Hove self-correlation function is related to both the observation window and the time scale of polymer dynamics. In flexible and semiflexible polymer solutions, diffusion is Fickian only if the particles are much smaller than the structural length scales in the network, but not if the nanoparticle size is comparable to or larger than the structural length scale of the network (Brochard Wyart & De Gennes, 2000). Although progress has been made in the conceptual description of time-dependent nanoparticle diffusion in flexible polymer networks (Cai *et al.*, 2011), these effects relate to (passive) microrheology and therefore fall outside the scope of this review. Here, we will focus on particle diffusion in rigid/fibrous networks (e.g. gels) where the ensemble-average propagator can be non-Gaussian and time-dependent, depending on network heterogeneity (Table 1, case C). In these systems, the van Hove self-correlation function can be non-Gaussian due to confinement of the nanoparticles within the micro- or mesoporous network, or due to heterogeneity (i.e. a large distribution of pore sizes) (Valentine *et al.*, 2001). If the nanoparticles are not confined and the pore size distribution is finite (i.e. the material is not "fractal" at all length scales) the propagator will always be Gaussian at long time scales (Netz & Dorfmüller, 1995; B. Wang *et al.*, 2012). In this case, the observed diffusion coefficient will be lower than in pure solvent and from this decrease, structural information about the network can be derived through physical models of diffusion in gels.

Two methodological approaches for probing biopolymer gels can be distinguished based on the characteristics of diffusional nanoparticles used. Nanoparticles can be either flexible and deformable when constrained to small volumes, or rigid and spherical. Models for both cases will be discussed in the following. For both types of nanoparticles, physico-chemical interactions between network and nanoparticles are not accounted for in diffusion models; In case of attractive interactions between particles and network, diffusion coefficients have been shown to be lower than predicted from physical models of diffusion (E. Schuster, Hermansson, Öhgren, Rudemo, & Lorén, 2014). Hence, for practical applications, nanoparticles need to be selected to be "non-sticky" with regard to the network.

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Diffusion models for flexible nanoparticles

Most applications of quantitative nanoparticle diffusometry use flexible polymers as diffusional probes. Flexible particles include dextran, a branched polysaccharide, and polyethylene glycol (PEG), a linear polymer. These polymers are random coils in dilute solutions, but deform in more crowded environments. The power law $D \propto M_w^{\alpha}$ gives the dependency of the diffusion coefficient of polymer chains in solution on their molecular weight M_w. The magnitude of α depends on the characteristics of the environment through which polymers diffuse. According to the Zimm model for diffusion of randomly coiled polymer chains in dilute solution, diffusion coefficients are inversely proportional to the size of the coil: $D \propto R^{-1}$. Flory predicted that $M_w^{\alpha} \propto R^{5/3}$ (fractal dimension 5/3) for swollen linear chains in dilute solution. It follows that α equals $-3/5$ in dilute solution. In crowded environments, de Gennes predicted reptation-like diffusion of polymer chains (i.e. snake-like movement), for which α equals -2 (Rubinstein & Colby, 2003). In reality, α often falls in-between these two situations, which could correspond to an incomplete deformation of chains into, for example, an ellipsoid shape (Fig. 1) (Favre, Leonard, Laurent, & Dellacherie, 2001). The molecular-weight dependency of diffusion coefficients of linear polymers can, therefore, give an estimate of the mesh size of the network. In case the polymer coil size surpasses the network mesh size, α shifts to more negative values.

We note that the term mesh size is poorly defined in most of the literature cited here. The definition depends fully on the context and the assumed structural model of the gel. Generally, the term seems to be used to describe a measure of the average distance between polymer strands or fibers, analogous to the correlation length in semidilute polymer solutions as defined by de Gennes (De Gennes, 1979). Sometimes, it is used to describe an average pore size, or another structural-model dependent length scale that can be used to describe the polymer network morphology as for example the particle-accessible volume term proposed by Babu *et al.* (Babu, Gimel, & Nicolai, 2008).

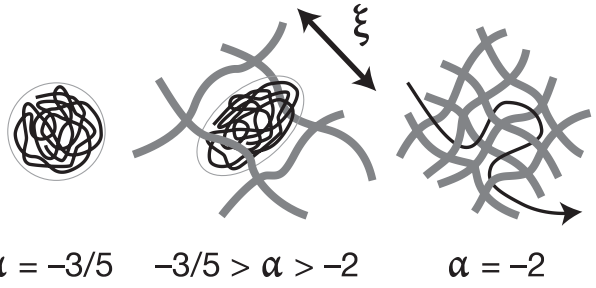

Fig. 1. The dependency of the diffusion coefficient D of a flexible nanoparticle (a polymer chain) on its molecular weight M_w changes according to the power law $D \propto M_w^{\alpha}$, where the magnitude α depends on the degree of crowding. In one limit, nanoparticles are random coils in dilute solution ($\alpha = -3/5$). Nanoparticles begin to deform when the mesh size ξ is of the same order as the radius of gyration of the nanoparticle polymer coil. When ξ is much smaller than the radius of gyration of the free polymer coil, unfolded chains must reptate through the crowded network ($\alpha = -2$). Reproduced from Favre *et al.* (2001), Copyright 2001, with permission from Elsevier.

Diffusion models for rigid, spherical nanoparticles

There is a large body of literature describing diffusion of rigid and spherical nanoparticles through polymer networks. Various rigid and spherical nanoparticles have been used, ranging from proteins (Silva, Peixoto, Lortal, & Floury, 2013) to solid gold colloidal nanoparticles (Kohli & Mukhopadhyay, 2012). A recently introduced class of spherical nanoparticles are dendrimers: macromolecules consisting of successive branched repeating units, which extend radially outward in up to ~10 consecutive layers or "generations", leading to particle diameters in the 10^0 nm range. Dendrimers are essentially monodisperse, because of controlled stepwise size increments during synthesis (Cheng, Prud'homme, & Thomas, 2002).

In order for the nanoparticles to be non-sticky with respect to biopolymer networks, they have to be charge-neutral and free of any chemical groups that can engage in strong specific or nonspecific interactions with the network. PEGylation of organic particles such as dendrimers is most often used for this purpose. Other water-soluble, charge-neutral coatings, such as poly(vinyl alcohol) (PVA) and dextran, have been described (A. K. Gupta & Gupta, 2005), but have not been applied yet as coatings for diffusional nanoparticles.

The degree of rigidity of nanoparticles can be expressed by their fractal dimension ν, defined by $M_w \propto R^{\nu}$, where R is nanoparticle size. Fractal dimension of a nanoparticle class can be used as a proxy for their conformation. For solid spheres, ν equals 3; for ideal chain random polymer coils, ν equals 2. For dextrans – slightly branched polysaccharides with an expanded conformation – ν equals 2.3. Dendrimer fractal dimensions fall in-between 2.3 and 3 (Cheng *et al.*, 2002).

There are many physical models that describe the diffusion of rigid and spherical nanoparticles in polymer networks as a function of structural parameters, such as nanoparticle radius, polymer volume fraction and polymer strand radius. Masaro and Zhu have reviewed different modeling approaches (Masaro & Zhu, 1999). In another review, Amsden validated models against literature data and presented a list of models that could be reconciled with most experimental data (Amsden, 1998b). Those models that allow the derivation of structural descriptors of polymer networks have been included in Table 2. Predicted diffusion coefficients should be interpreted as long-time diffusion coefficients. All models are based on the "obstruction effect"; some also include hydrodynamic arguments and generally apply only to rigid networks.

Obstruction effect models rely on the notion that the volume occupied by the rigid polymer network is inaccessible to the diffusing species, leading to an increased path length between two points in the system (Fig. 2).

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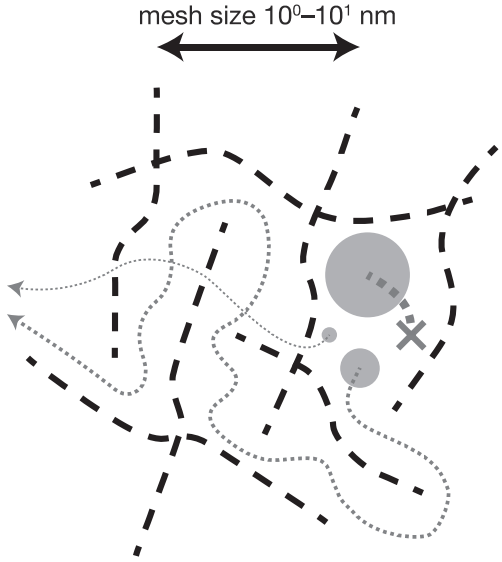

Fig. 2. Illustration of the obstruction effect (tortuosity) in a rigid/fibrous network. Black, dashed lines indicate polymer strands. Gray, dotted lines indicate diffusion trajectories. The smallest (nano)particles can diffuse nearly freely between two points in the system, whereas intermediate-size particles have to diffuse around the barriers to reach the same end-point. The largest particles are trapped. Cf. Table 2 for obstruction-effect models for diffusion in rigid/fibrous polymer networks.

This leads to a decrease of the nanoparticle diffusion coefficient at long time scales, with respect to the diffusion coefficient in solvent. The factor with which the diffusion coefficient is reduced is often reported as the tortuosity (L. M. Sanders & Hendren, 1997). In a system with the same mesh size, larger particles will experience a larger obstruction effect than smaller particles. The approach can be used only when the presumption of a motionless network holds, and is therefore particularly useful in rigid/fibrous networks (Ogston, 1958). Besides this, obstruction models obviously break down if diffusants are too large, so that hydrodynamic drag with polymers increases significantly or that diffusants are immobilized through the "sieving" action of the polymer mesh (Lauffer, 1961). The obstruction effect also lends itself to be predicted as a function of any random structure using Monte Carlo simulations (Babu *et al.*, 2008; Kvarnström, Westergård, Lorén, & Nydén, 2009). It can be seen in Table 2 that obstruction-effect based models mainly predict polymer strand radii. The combination of polymer strand radius and polymer volume fraction can be used to estimate a mesh size.

In polymer networks, not only the obstruction effect, but also hydrodynamic drag forces play an important role in determining particle dynamics, and therefore models based purely on the obstruction effect are an oversimplification. Consider a nanoparticle that has to pass through a narrow pore: here, displacement of the constituent fluid is obviously more difficult than in bulk solution. Hydrodynamic approaches aim to predict the magnitude of the drag coefficient f in the Einstein–Smoluchowski equation. Hydrodynamic models take into account the friction between nanoparticles and polymers, between nanoparticles and the solvent and between polymers and solvent.

Other forces present in colloidal systems such as electrostatic interactions and depletion forces (Asakura & Oosawa, 1954) are generally ignored in diffusion models for rigid/fibrous networks. The use of particles that are not solvated can lead to slippage and a violation of no-slip boundary conditions, and can compromise the validity of hydrodynamical models.

Instrumental methods

In order to prevent perturbation of the biopolymer network nanoparticles need to be dosed at low levels, typically at 0.1–1 % (w/w). These low levels impose demands on the experimental method in terms of sensitivity. Furthermore, strong background signals of the matrix require selectivity with which nanoparticle signals can be detected. We will briefly discuss four suitable instrumental methods that meet these demands: fluorescence recovery after photobleaching (FRAP) microscopy, fluorescence correlation spectroscopy (FCS), single-particle tracking (SPT), and pulsed-field gradient nuclear magnetic resonance (PFG NMR) spectroscopy. An overview of these methods, including the time and length scales that can be probed and the required nanoparticle properties, can be found in Table 3.

FRAP is a non-invasive method that can quantify the apparent diffusion coefficient of fluorescent-labeled particles as well as the mobile and immobile fractions. A laser is used to give a short, high-intensity pulse to irreversibly bleach fluorescent labeled particles in a ~micrometer-size spot in the sample. Subsequently, a time trace of the *in situ* recovery of fluorescence is recorded, the recovery being due to non-bleached fluorescent particles outside the spot exchanging with bleached particles within the bleached spot. FRAP in conjunction with confocal laser scanning microscopy (CLSM) allows *in situ* selection of the bleaching spot. Micromolar fluorescent particle concentrations already suffice. Using models that account for the outflux of bleached probes and the influx of fluorescent probes, a diffusion coefficient can be extracted from image series recorded during fluorescence recovery. The accuracy of the FRAP method therefore ultimately depends on validity of the model used (Axelrod *et al.*, 1976; Braeckmans *et al.*, 2003). A single diffusion coefficient can be determined to with a precision of ~1%. In case of bimodal diffusion, diffusion coefficients can be extracted with an error of ~10% (P. Jönsson, Jonsson, Tegenfeldt, & Höök, 2008).

FCS uses a confocal laser microscopy setup to monitor the fluctuations of fluorescence due to diffusion of fluorescently labeled particles inside a sub-micrometer confocal volume (~1 μm^3) (Rathgeber *et al.*, 2009). Brownian

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motion of fluorescent molecules in and out of the laser confocal volume will lead to fluorescence intensity fluctuations. From the fluorescence-intensity autocorrelation function, the particles' diffusion coefficients —also if the diffusion is multi-modal— or the time-dependent mean-square displacements can be calculated (Koynov & Butt, 2012; Rathgeber *et al.*, 2009). FCS is a highly sensitive and selective technique that can be used on very small volumes, but careful calibration is required, because the volume of the laser focal point may vary depending on diffractive index. For imaging purposes, the method can be used in scanning mode (sFCS) (Petrášek, Ries, & Schwille, 2010). FCS is sensitive and can measure self-diffusion over a wide range of observation times (10^{-7}–10^{2} s), but ideally the samples should have a high degree of optical transparency. Like FRAP, FCS requires tracers functionalized with a fluorescent dye. An analogous method is dynamic light scattering (DLS), which measures the fluctuation of light that is scattered by diffusing particles. Recent developments in extending the lower particle size limit of DLS (Takahashi, Kato, & Kinugasa, 2011) are difficult to exploit due to background scattering of other mobile species in the matrix, which complicates the interpretation of correlation curves.

SPT provides a direct visualization of individual diffusive trajectories of particles. So far, SPT has mainly been used for microrheology experiments, in which the micron-size tracer particles are visible under an optical microscope (Dickinson, 2011). Tracking of sub-micron particles, on the other hand, can be achieved by either detecting scattered light (Filipe *et al.*, 2010), or fluorescence (Braeckmans *et al.*, 2010; Mun *et al.*, 2014). The observation of nanoparticles based on laser diffraction is only possible in systems where no other scattering elements are present. This is not a likely situation for most food systems, hence tracking of fluorescent particles is more promising for this purpose. The smallest fluorescent particles that have been tracked were 50 nm in diameter. A sensitive technique to detect motion of even smaller fluorescent particles is total internal reflection (TIRF), which has, however, a rather low sample penetration depth (Zareh, DeSantis, Kessler, Li, & Wang, 2012). This may compromise representative sampling of diffusive trajectories in heterogenous systems and limit the time window of the method. SPT is one of two methods discussed here that allow direct assessment of the distribution of particle displacements in an ensemble, the other one being PFG NMR. Most applications of SPT pertain to assessment of size, concentration and functionality of nanoparticles; only few examples have been given to probe local sub-micron network structure and dynamics (Mun *et al.*, 2014). To our knowledge no application has appeared to assess sub-micron food structures.

PFG NMR is a method by which time-dependent displacements of molecular ensembles can be determined from the dephasing and rephasing of molecular spin magnetization by pulsed magnetic field gradients. These experiments can be implemented on any NMR spectrometer equipped with a field gradient coil. PFG-based methods are particularly powerful if it comes to nanoparticle diffusometry in materials that are not optically transparent: NMR uses radiofrequency waves, which also penetrate optically non-transparent materials. The method is therefore well suited for nanoparticle diffusometry in optically non-transparent polysaccharide and protein matrices.

Using PFG NMR, the time-dependent diffusion coefficient can be determined in the 10^{0}–10^{3} ms range. By varying the observation time, the distance over which the network is "probed" can therefore be changed (Callaghan, 2011). In an experiment in which nanoparticles with different sizes (and hence, diffusion coefficients) are used to probe a network, the diffusion time can be adjusted to make sure that the mean-square displacement of different particles is identical. More specifically, PFG NMR directly detects the particles' van Hove self-correlation function. This means that multi-modal diffusion can be observed. Although no information about individual particles is retained, as is the case in SPT, the ensemble average of displacements over all particles in the sample is obtained (Callaghan, 2011). Only with PFG NMR, the diffusion of the ensemble of nanoparticles in a sample can be observed in a single experiment, which is a particular advantage for probing heterogenous systems. Detection by NMR allows for separation signals on the basis of chemical shift or transverse relaxation time T_2, which offers the opportunity to resolve signals of nanoparticles and matrix (biopolymer network, solvent, solutes) (Lorén *et al.*, 2009; de Kort, van Duynhoven, Hoeben, Janssen, & Van As, 2014). The frequency domain NMR experiment that separates molecular components on the basis of chemical shift is termed "diffusion-ordered spectroscopy" (DOSY), the time domain experiment that separates components on the basis of T_2 is termed "diffusion–relaxation correlation spectroscopy" (DRCOSY) (Callaghan, 2011). Diffusion measurements can also be performed in MRI (imaging) mode. This allows the determination of local diffusion coefficients on the micron scale, with a field of view of 10^{-2}–10^{-1} m, depending on the setup. This is what makes PFG NMR a truly multi-length-scale method. With an MRI setup, also the anisotropic diffusion tensor can be resolved (Future Trends) (Callaghan, 2011).

Applications in food science

In Table 4, a range of recent applications of nanoprobe diffusometry in food biopolymer networks has been collected. Early contributions introducing the concepts of nanoparticle diffusometry in biopolymer networks stem from the 80s. For example, Brown *et al.* used PFG NMR to study self-diffusion of PEG nanoparticles in dextran solutions, and of small molecules (ethylene glycol, crown ether) in cellulose dispersions (Brown & Stilbs, 1983; Brown, Stilbs, & Lindström, 1984). The concept of

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Table 4. Overview of applications of nanoparticle diffusometry in food related materials. The table includes systems studied, and instrumental methods and nanoparticles used. Also modeling approaches are indicated, which can be either based on the obstruction effect (in case of rigid nanoparticles, cf. also Table 2) or on power laws (in case of flexible nanoparticles). It is also indicated if a model-free interpretation was made, a new model was proposed or models were used that have not been discussed in this paper (because they do not model nanoparticle diffusion in terms of structural network parameters).

Biopolymer network	Instrumental method	Nanoparticles (hydrodynamic radius R_h or molecular weight M_w)	Model class	References
Agarose	refractive-index method (see in ref.)	PEG[a] (M_w = 0.2, 0.6, 1, 10 kDa)	power-law, obstruction effect	(Weng, Liang, Zhang, Zhang, & Xu, 2005)
Alginate	release kinetics (see (Favre *et al.*, 2001)), PFG NMR[b]	star-shaped (M_w = 24 kDa) and linear (M_w = 80–750 kDa) PEG, dendrimers (R_h = 2–8 nm)	hydrodynamics modeling, obstruction effect	(Baldursdóttir, Kjøniksen, & Nyström, 2006; Bernin *et al.*, 2011; Favre *et al.*, 2001)
Beta-lactoglobulin	CLSM[c] FRAP[d], PFG NMR	fluorescent-labeled dextran (R_h = 5.5, 23 and 50 nm), dextran (R_h = 8, 11, 23 nm)	model-free interpretation	(Balakrishnan, Nicolai, & Durand, 2012; Croguennoc, Nicolai, Kuil, & Hollander, 2001)
Casein micelles and caseinate	PFG NMR	linear PEG (M_w = 1–600 kDa), dendrimers (R_h = 2–6 nm)	obstruction effect, power law	(Colsenet, Mariette, & Söderman, 2006; Colsenet, Söderman, & Mariette, 2005a, 2005b; Le Feunteun & Mariette, 2007, 2008a, 2008b, Le Feunteun, Ouethrani, & Mariette, 2012; Salami, Rondeau-Mouro, Barhoum, van Duynhoven, & Mariette, 2014; Salami, Rondeau-Mouro, van Duynhoven, & Mariette, 2013a, 2013b)
Curdlan	PFG NMR	PEG (M_w = 1, 3 kDa), micelles (R_h = 41 nm)	models by Cukier (Cukier, 1984) and Petit (Petit, Roux, Zhu, & Macdonald, 1996)	(Kwak & Lafleur, 2003b)
Collagen	(line-)FRAP	FITC-dextran (R_h = 20 nm)	model for anisotropic diffusion in aligned-fiber systems	(Stylianopoulos, Diop-Frimpong, Munn, & Jain, 2010)
Dextran	FRAP, PFG NMR	fluorescein-labeled dextran (M_w = 40, 150 kDa), fluorescein-labeled polystyrene spheres (R_h = 19 nm), and SPS, CPC, and Triton X-100 micelles (R_h = 17, 29 53 nm)	discussion of hydrodynamic models, scaling laws	(Furukawa, Arauz-Lara, & Ware, 1991; Kwak & Lafleur, 2003a)
Gelatin	CLSM FRAP	FITC[e]-dextran (R_h = 6 and 34 nm)	model-free interpretation	(Hagman, Lorén, & Hermansson, 2010)
Guar galactomannan	FRAP	FITC-dextran (M_w = 20, 40, 71 kDa), dextran and dendrimers, R_h = 1–20 nm	models by Cukier (Cukier, 1984), Langevin (Langevin & Rondelez, 1978) and Altenberger (Altenberger, Tirrell, & Dahler, 1986)	(Burke, Park, Srinivasarao, & Khan, 2000; Cheng *et al.*, 2002)
Kappa-carrageenan	PFG NMR, CLSM FRAP	PAMAM and PEGylated, ^{19}F-labeled PPI dendrimers (R_h = 2–8 nm), FITC-dextran (10, 500 kDa)	model-free interpretation	(Hagman, Lorén, & Hermansson, 2012; de Kort *et al.*, 2014; Lorén *et al.*, 2009)
Konjac glucomannan	FRAP	FITC-dextran (M_w = 77, 130, 511, 2000 kDa)	power law	(Alvarez-Manceñido *et al.*, 2006)
Pig gastric mucin	PFG NMR, DLS[f]	linear PEG (M_w = 1, 6, 59, 716 kDa), polysorbate 80 (R_h = 6 nm), polystyrene beads (R = 54 nm)	hydrodynamics, power law, model-free interpretation	(Celli *et al.*, 2005; Lafitte, Söderman, *et al.*, 2007; Lafitte, Thuresson, *et al.*, 2007)
Starch	FRAP	dextran (M_w = 70, 250 kDa)	power law	(Perry, Fitzgerald, & Gilbert, 2006)

Ultrafiltrated milk model cheese	CLSM FRAP	FITC-dextran (R_h = 2−24 nm) and FITC-dairy proteins (R_h = 2−4 nm)	model-free interpretation	(Floury, Madec, Waharte, Jeanson, & Lortal, 2012; Silva *et al.*, 2013)
Whey protein	PFG NMR	linear PEG (M_w = 1, 9, 82 kDa)	power law	(Colsenet, Söderman, & Mariette, 2006)
Whey protein isolate−gellan gum, micro-phase-separated system	PFG NMR, FRAP CLSM	alpha-lactalbumin (R_h = 2 nm), FITC-dextran (M_w = 10 kDa)	Lattice Boltzmann simulations in model gel	(Wassén *et al.*, 2014)

[a] PEG = Polyethylene glycol.
[b] PFG NMR = Pulsed-field gradient nuclear magnetic resonance.
[c] CLSM = Confocal laser scanning microscopy.
[d] FRAP = Fluorescence recovery after photobleaching.
[e] FITC = Fluorescein isothiocyanate, a fluorescent dye.
[f] DLS = Dynamic light scattering.

inferring information about the biopolymer network from the diffusion of nanoparticles was introduced here, and simple obstruction effect-based models were used to describe the data. At the time of these studies, however, models of diffusion that included hydrodynamic interactions were not available. Also, no conceptual distinction was made between diffusometry in solutions of flexible polymers and rigid/fibrous networks or gels, which relates to the fact that the microrheological experiment originated only a decade later. The first steps toward the use of FRAP for nanoparticle diffusometry stems from the early 90s; significant work includes that of Furukawa *et al.*, describing nanoparticle diffusion in dextran solutions, although this study was not directly related to foods (Furukawa *et al.*, 1991). The work already includes a critical discussion of the meaning of nanoparticle diffusometric data in polymer solutions using reptation and hydrodynamic concepts.

One of the first applications in food biopolymer networks is an FRAP study on a guar galactomannan model system using FITC-dextran complexes (Burke *et al.*, 2000). The interpretation of the diffusometric data related to the crossover point between Zimm and reptation dynamics in dilute and concentrated polymer solutions, respectively, as described e.g. by Nydén and Söderman (Nydén & Söderman, 1998). Soon thereafter, Favre *et al.* published the first PFG NMR study of PEG diffusion in alginate gels, applying these concepts to diffusion of flexible probes in dilute versus concentrated systems (Favre *et al.*, 2001). We have described this approach, which is still current, in the section about diffusion models for flexible nanoparticles. Over the following decade, many more studies in both polysaccharide and protein systems would follow, with the FRAP and PFG NMR methods as workhorses. During this period, the diversity in nanoparticles increased. In 2003, Kwak and Lafleur used spherical particles (micelles) for the first time for the characterization of a biopolymer network by PFG NMR (Kwak & Lafleur, 2003b). The first foods-related diffusion study using dendrimers would follow some years later in 2009 (Lorén *et al.*, 2009). We will now describe the most important diffusometry studies in polysaccharide and protein networks.

Characterization of polysaccharide networks

Baldursdóttir studied the effect of photo-degradation of alginate gels on diffusion of embedded PEG molecules and was able to infer microstructural changes within the gels from the changes in diffusive behavior of nanoparticles (Baldursdóttir *et al.*, 2006). Bernin *et al.* characterized these systems using a combination of transmission electron microscopy (TEM) and nanoparticle diffusometry. They calculated alginate polymer-strand radii from the reduced diffusion coefficient of dendrimer nanoparticles and validated this information against TEM micrographs. They found that in some gels, a submicron network was present that TEM was unable to visualize (Bernin *et al.*, 2011). Kappa-carrageenan gels have also been analyzed with the

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combination of TEM and nanoparticle diffusometry. Using TEM, the effects of polymer concentration and different salt conditions on microstructure were visualized. Not only polymer density was found to affect nanoparticle self-diffusion; particularly salt conditions led to interesting submicron-scale changes in network heterogeneity, which were reflected by changes in diffusion behavior of the nanoparticles. In some cases, bi-modal diffusion was observed, tentatively due to the presence of two different microdomains within the gel morphology (de Kort *et al*., 2014; Lorén *et al*., 2009).

Characterization of protein networks

Croguennoc and later Balakrishnan studied nanoparticle diffusion during and after gelation of beta-lactoglobulin, a globular protein that is the major component of whey, and which can form gels through heating. These studies showed that nanoparticles could be used to assess the kinetics of the gelation process, and the profound effects of protein and salt concentrations on void sizes in resulting gels, which was reflected by changes in nanoparticle diffusion coefficients (Balakrishnan *et al*., 2012; Croguennoc *et al*., 2001).

In order to see the effect of fiber alignment, aligned collagen systems were studied using FITC-labeled dextran molecules (Stylianopoulos *et al*., 2010). A model was derived in order to understand the relationship between collagen fiber radius and particle diffusion anisotropy. It was concluded that material anisotropy could only be observed with nanoparticles sufficiently large with respect to the aligned-fiber radius. In this study, anisotropy was indeed observed when the particle-fiber radius ratio was between 1 and 2, whereas in an earlier study in which the ratio was $\ll 1$, isotropic diffusion was observed.

Mariette *et al*. have intensively studied coagulation of milk proteins into casein micelle gels using the nanoparticle diffusometry approach. Their initial work focused on the diffusion of flexible PEG molecules through the networks (Colsenet, Mariette, *et al*., 2006; Colsenet, Söderman, & Mariette, 2005a, 2005b; Le Feunteun & Mariette, 2007; 2008a). More recently, dendrimer particles have been used for the same purpose (Salami *et al*., 2014; Salami, Rondeau-Mouro, van Duynhoven, & Mariette, 2013b). Le Feunteun *et al*. deployed NMR diffusometry to continuously monitor the changes in the structure during the coagulation process itself (Le Feunteun & Mariette, 2008b). The PEG-probe based approach was also used for the characterization of whey protein gels (Colsenet, Söderman, *et al*., 2006).

In a more recent approach, Le Feunteun *et al*. and Salami *et al*. followed diffusion of casein proteins through porous micellar aggregates. Diffusion was described by the Speedy model for hard-sphere diffusion through voids in a system of closely packed spheres. This way, the voluminosity of the casein micelles could be derived (Le Feunteun *et al*., 2012; Salami, Rondeau-Mouro, van Duynhoven, & Mariette, 2013a).

In very recent work, Floury and Silva have studied the diffusion of solutes and nanoparticles through a model cheese, based on ultra-filtrated milk. Understanding mass transport in cheese is relevant, because diffusion of salt, moisture and metabolites is very important for the final quality of cheese. Particularly the diffusion of nanoscopic particles in cheese had not yet been studied. This example illustrates how the step from abstract model systems to more complex and heterogenous systems is being taken (Floury *et al*., 2012; Silva *et al*., 2013).

Future trends

From the food-related application areas of nanoparticle diffusometry that have been explored in the last decade, it has become clear that particularly rigid biopolymer networks (gels) that are structured on the submicron scale lend themselves for characterization using nanoparticle diffusometry. Using obstruction models it is possible to derive quantitative structural parameters in a straightforward manner, which is a considerable advantage over current microscopic methods. We will now sketch some perspectives for the characterization of rigid as well as flexible polymer networks. We will also discuss the possibility to use nanoparticle diffusometry to measure network anisotropy and the scope of novel nanoparticle designs.

Shear-induced microstructural anisotropy

Since nanoparticle diffusion in rigid systems can be predicted from the obstruction effect, it should also be sensitive to network anisotropy. For example, in systems of aligned fibers, diffusion in the parallel direction should be less hindered than in the orthogonal direction. Such effects have been observed for nanoparticle diffusion in aligned collagen fibers in cartilage by line-FRAP (Stylianopoulos *et al*., 2010). Also SPT and PFG NMR/MRI should be able to observe anisotropy, because these methods can be used to observe diffusion in different orientations. The approach should also work for shear-induced network anisotropy in food systems. The approach can be combined with *in-situ* rheological experiments in an MRI setup (rheo-NMR) (Callaghan, 2011). Lutti and Callaghan showed that it is possible to measure particle diffusion also under dynamic (shear) conditions by MRI (Lutti & Callaghan, 2006).

Design of functionalized nanoparticles

Obstruction models generally assume the diffusing species to be rigid and spherical. In this light, dendrimers are near-ideal diffusional nanoparticles because they have a fractal index close to that of solid spheres and are essentially monodisperse. Their size can be varied in several discrete steps up to several nanometers. An additional attractive feature is their well-defined number of end groups, which in principle can be readily functionalized.

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This way, their surface chemistry (including surface charge) can be fine-tuned to specific applications, e.g. by PEGylation in order to better satisfy the non-sticky condition. In case neutral particles are required, their charged surface groups should be functionalized with neutral end groups. For example, the commercially available poly(amido amine) (PAMAM) and poly(propylene imine) (PPI) dendrimers have primary amine end groups and can therefore carry charges (Boas, Christensen, & Heegaard, 2006).

Additionally, dendrimers can be functionalized with labels, such as fluorescent dyes to allow detection by FRAP, FCS and SPT. We recently reported a design of (covalently) PEGylated, charge neutral, ^{19}F-labeled PPI dendrimers for (background-free) observation by PFG NMR (de Kort *et al*., 2014). A limitation of dendrimers is their limited size range (max. diameter ~10 nm) and relatively high cost. Bourouina *et al*. recently circumvented this drawback by using complex coacervate (polyion) micelles as a chassis for designing and manufacturing larger and functionalized diffusional nanoparticles (Bourouina, Cohen Stuart, & Kleijn, 2014).

Micron-scale heterogeneity

Obstruction-effect based modeling of diffusional behavior of nanoparticles can yield quantitative network descriptors in a straightforward manner. These models assume that a single diffusion coefficient is found for the diffusing species. Often, systems are not homogenous on the micron-scale, meaning that on time scales at which mean particle displacement is on the order of the length scale of the heterogeneities, a spatial distribution of nanoparticle diffusion coefficients will be present. The spatial distribution arises from the different microenvironments through which the nanoparticles move. In the long run, if nanoparticles can move between microenvironments, a single, average "terminal" diffusion coefficient will be observed (central limit theorem, cf. Table 1). The observation time at which the distribution of diffusion coefficients disappears, and only a single nanoparticle diffusion coefficient remains, is therefore directly related to the size of the heterogeneities in the structure. If no exchange between domains is possible, increasing diffusion time will not lead to a single diffusion coefficient, and the distribution in particle displacements will be related to domain sizes.

Micron-size tracer particles have been used to assess heterogeneity of agarose (Valentine *et al*., 2001), acid milk gels (Cucheval, Vincent, Hemar, Otter, & Williams, 2009) and phase-separated systems (Dickinson, Murray, & Moschakis, 2007). *Nanoparticles* have been used to observe heterogeneity in kappa-carrageenan gels (de Kort *et al*., 2014; Lorén *et al*., 2009) Multi-modal diffusion can be observed using PFG NMR, since this method measures the ensemble-average propagator directly.

PFG NMR offers a broader perspective on gel heterogeneity, because the diffusion-observation time can be varied, opening the opportunity to monitor the changes in diffusion coefficients as a function time. This would allow investigation of the nature of the heterogeneities, that is, whether or not exchange between the domains takes place, and derivation of structural parameters. Diffusion–diffusion exchange spectroscopy (DEXSY) allows a more direct assessment of diffusive exchange between two nanoparticle populations (Callaghan, 2011).

Despite its promise, we have not been able to find any food science related studies that follow this approach, possibly because of the limited time-observation window of NMR (10^0–10^3 ms).

Changes in sub-micron structure under dynamical conditions

Most diffusometric studies summarized in Table 4 focus on the characterization of network microstructure under static conditions. In casein systems, microstructural changes have been studied under dynamical conditions. Le Feunteun *et al*. have monitored the evolution of self-diffusion coefficients of PEG as a function of pH during acid coagulation, during chymosin-induced coagulation and during combined acid-enzyme coagulation. PEG diffusion revealed many of the structural changes occurring during the coagulation process. The information obtained from PEG diffusion was found to be highly complementary with rheological data (Le Feunteun & Mariette, 2008a; 2008b).

Besides monitoring gelation processes, also other structural transitions, such as syneresis and retrogradation, can be monitored in a time-dependent and non-invasive manner at sub-micron scale.

Conclusion

In food science, nanoparticle diffusometry has been mainly used for the characterization of heterogenous and rigid polymer networks. The theoretical framework for interpreting diffusion data is becoming more elaborate and mature. Two different modeling approaches can be distinguished. (1) Models that predict the diffusion of rigid, spherical nanoparticles through polymer gels. These models are mainly based on the notion of the obstruction effect and can be used to predict structural network descriptors, such as mesh size and average polymer strand radii. (2) Models that describe the diffusion of flexible nanoparticles such as polymer chains in crowded systems, based on arguments from polymer theory. These models assume that the diffusion of nanoparticles changes from Zimm-like to reptation-like, depending on the degree of crowding. By varying the molecular weight of the nanoparticle, information about the microstructure (mesh size) can be obtained.

This review aimed at summarizing the theoretical framework and methodological approaches for nanoparticle diffusometry. The method is already being used to characterize more advanced food matrices, such as model cheeses (Silva *et al*., 2013) More exciting developments are expected, such as functionalized nanoparticle designs.

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Furthermore we expect to see more work relating to the observation of changing diffusion coefficients under dynamical conditions, the observation of micron-scale heterogeneity from multi-modal diffusion of nanoparticles and the observation of shear-induced network anisotropy.

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References

Altenberger, A. R., Tirrell, M., & Dahler, J. S. (1986). Hydrodynamic screening and particle dynamics in porous media, semidilute polymer solutions and polymer gels. *The Journal of Chemical Physics, 84*(9), 5122–5130. http://dx.doi.org/10.1063/1.450665.

Alvarez-Manceñido, F., Braeckmans, K., De Smedt, S. C., Demeester, J., Landin, M., & Martínez-Pacheco, R. (2006). Characterization of diffusion of macromolecules in konjac glucomannan solutions and gels by fluorescence recovery after photobleaching technique. *International Journal of Pharmaceutics, 316*(1–2), 37–46. http://dx.doi.org/10.1016/j.ijpharm.2006.02.029.

Amsden, B. (1998a). Solute diffusion in hydrogels. *Polymer Gels and Networks, 6*(1), 13–43. http://dx.doi.org/10.1016/S0966-7822(97)00012-9.

Amsden, B. (1998b). Solute diffusion within hydrogels. Mechanisms and models. *Macromolecules, 31*(23), 8382–8395. http://dx.doi.org/10.1021/ma980765f.

Asakura, S., & Oosawa, F. (1954). On interaction between two bodies immersed in a solution of macromolecules. *The Journal of Chemical Physics, 22*(7), 1255–1256. http://dx.doi.org/10.1063/1.1740347.

Axelrod, D., Koppel, D. E., Schlessinger, J., Elson, E., & Webb, W. W. (1976). Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. *Biophysical Journal, 16*(9), 1055–1069. http://dx.doi.org/10.1016/S0006-3495(76)85755-4.

Babu, S., Gimel, J.-C., & Nicolai, T. (2008). Tracer diffusion in colloidal gels. *The Journal of Chemical Physics, 112*(3), 743–748. http://dx.doi.org/10.1021/jp076342.

Balakrishnan, G., Nicolai, T., & Durand, D. (2012). Relation between the gel structure and the mobility of tracers in globular protein gels. *Journal of Colloid and Interface Science, 388*(1), 293–299. http://dx.doi.org/10.1016/j.jcis.2012.08.032.

Baldursdóttir, S. G., Kjøniksen, A.-L., & Nyström, B. (2006). The effect of riboflavin-photoinduced degradation of alginate matrices on the diffusion of poly(oxyethylene) probes in the polymer network. *European Polymer Journal, 42*(11), 3050–3058. http://dx.doi.org/10.1016/j.eurpolymj.2006.08.011.

Bernin, D., Goudappel, G.-J., van Ruijven, M., Altskär, A., Ström, A., Rudemo, M., et al. (2011). Microstructure of polymer hydrogels studied by pulsed field gradient NMR diffusion and TEM methods. *Soft Matter, 7*(12), 5711–5716. http://dx.doi.org/10.1039/c1sm05070b.

Boas, U., Christensen, J. B., & Heegaard, P. M. H. (2006). Dendrimers: design, synthesis and chemical properties. *Journal of Materials Chemistry, 16*(38), 3785–3798. http://dx.doi.org/10.1039/B611813P.

Bourouina, N., Cohen Stuart, M. A., & Kleijn, J. M. (2014). Complex coacervate core micelles as diffusional nanoprobes. *Soft Matter*, . http://dx.doi.org/10.1039/C3SM52245H.

Braeckmans, K., Buyens, K., Naeye, B., Vercauteren, D., Deschout, H., Raemdonck, K., et al. (2010). Advanced fluorescence microscopy methods illuminate the transfection pathway of nucleic acid nanoparticles. *Journal of Controlled Release, 148*(1), 69–74. http://dx.doi.org/10.1016/j.jconrel.2010.08.029.

Braeckmans, K., Peeters, L., Sanders, N. N., De Smedt, S. C., & DeMeester, J. (2003). Three-dimensional fluorescence recovery after photobleaching with the confocal scanning laser microscope. *Biophysical Journal, 85*(4), 2240–2252. http://dx.doi.org/10.1016/S0006-3495(03)74649-9.

Brochard Wyart, F., & De Gennes, P. G. (2000). Viscosity at small scales in polymer melts. *The European Physical Journal E, 1*, 93–97.

Brown, W., & Stilbs, P. (1983). Self-diffusion of poly(ethylene oxide) in aqueous dextran solutions measured using FT-pulsed field gradient n.m.r. *Polymer, 24*(2), 188–192. http://dx.doi.org/10.1016/0032-3861(83)90131-3.

Brown, W., Stilbs, P., & Lindström, T. (1984). Self-diffusion of small molecules in cellulose gels using FT-pulsed field gradient NMR. *Journal of Applied Polymer Science, 29*(3), 823–827. http://dx.doi.org/10.1002/app.1984.070290310.

Burke, M. D., Park, J. O., Srinivasarao, M., & Khan, S. A. (2000). Diffusion of macromolecules in polymer solutions and gels: a laser scanning confocal microscopy study. *Macromolecules, 33*(20), 7500–7507. http://dx.doi.org/10.1021/ma000786l.

Cai, L.-H., Panyukov, S., & Rubinstein, M. (2011). Mobility of nonsticky nanoparticles in polymer liquids. *Macromolecules, 44*(19), 7853–7863. http://dx.doi.org/10.1021/ma201583q.

Callaghan, P. T. (2011). *Translational dynamics & magnetic resonance*. New York: Oxford University Press.

Carvajal-Rondanelli, P. A., & Lanier, T. C. (2010). Diffusion of active proteins into fish meat to minimize proteolytic degradation. *Journal of Agricultural and Food Chemistry, 58*(9), 5300–5307. http://dx.doi.org/10.1021/jf903580t.

Celli, J., Gregor, B., Turner, B., Afdhal, N. H., Bansil, R., & Erramilli, S. (2005). Viscoelastic properties and dynamics of porcine gastric mucin. *Biomacromolecules, 6*(3), 1329–1333. http://dx.doi.org/10.1021/bm0493990.

Cheng, Y., Prud'homme, R. K., & Thomas, J. L. (2002). Diffusion of mesoscopic probes in aqueous polymer solutions measured by fluorescence recovery after photobleaching. *Macromolecules, 35*(21), 8111–8121. http://dx.doi.org/10.1021/ma0107758.

Clague, D. S., & Phillips, R. J. (1996). Hindered diffusion of spherical macromolecules through dilute fibrous media. *Physics of Fluids, 8*(7), 1720–1731. http://dx.doi.org/10.1063/1.868884.

Colsenet, R., Mariette, F., & Söderman, O. (2006). *NMR study of the polyethyleneglycols diffusion in casein suspensions and gels.* Presented at the ISFRS 2006, 4th International symposium on food rheology and structure.

Colsenet, R., Söderman, O., & Mariette, F. (2005a). Diffusion of polyethyleneglycols in casein solutions and gels as studied by pulsed field gradient NMR. *Magnetic Resonance Imaging, 23*(2), 347–348. http://dx.doi.org/10.1016/j.mri.2004.11.049.

Colsenet, R., Söderman, O., & Mariette, F. (2005b). Effect of casein concentration in suspensions and gels on Poly(ethylene glycol)s NMR self-diffusion measurements. *Macromolecules, 38*(22), 9171–9179. http://dx.doi.org/10.1021/ma051294h.

Colsenet, R., Söderman, O., & Mariette, F. (2006). Pulsed field gradient NMR study of Poly(ethylene glycol) diffusion in whey protein solutions and gels. *Macromolecules, 39*(3), 1053–1059. http://dx.doi.org/10.1021/ma0519922.

Croguennoc, P., Nicolai, T., Kuil, M. E., & Hollander, J. G. (2001). Self-diffusion of native proteins and dextran in heat-set globular protein gels. *The Journal of Chemical Physics, 105*(24), 5782–5788. http://dx.doi.org/10.1021/jp0043726.

Cucheval, A. S. B., Vincent, R. R., Hemar, Y., Otter, D., & Williams, M. A. K. (2009). Multiple particle tracking investigations of acid milk gels using tracer particles with designed surface chemistries and comparison with diffusing wave spectroscopy studies. *Langmuir, 25*(19), 11827–11834. http://dx.doi.org/10.1021/la901424w.

Please cite this article in press as: de Kort, D. W., et al., Nanoparticle diffusometry for quantitative assessment of submicron structure in food biopolymer networks: A review, Trends in Food Science & Technology (2014), http://dx.doi.org/10.1016/j.tifs.2014.11.003

Cukier, R. I. (1984). Diffusion of Brownian spheres in semidilute polymer solutions. *Macromolecules, 17*(2), 252–255. http://dx.doi.org/10.1021/ma00132a023.

De Gennes, P. G. (1979). *Scaling concepts in polymer science*. Itaca: Cornell University Press.

Dickinson, E. (2011). Food colloids research: historical perspective and outlook. *Advances in Colloid and Interface Science, 165*(1), 7–13. http://dx.doi.org/10.1016/j.cis.2010.05.007.

Dickinson, E., Murray, B. S., & Moschakis, T. (2007). Particle tracking as a probe of microrheology in food colloids. In *Food colloids* (pp. 305–318). Cambridge: Royal Society of Chemistry. http://dx.doi.org/10.1039/9781847557698-00303.

Favre, E., Leonard, M., Laurent, A., & Dellacherie, E. (2001). Diffusion of polyethyleneglycols in calcium alginate hydrogels. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 194*(1), 197–206. http://dx.doi.org/10.1016/S0927-7757(01)00789-0.

Filipe, V., Hawe, A., & Jiskoot, W. (2010). Critical evaluation of nanoparticle tracking analysis (NTA) by nanosight for the measurement of nanoparticles and protein aggregates. *Pharmaceutical Research, 27*(5), 796–810. http://dx.doi.org/10.1007/s11095-010-0073-2.

Floury, J., Madec, M. N., Waharte, F., Jeanson, S., & Lortal, S. (2012). First assessment of diffusion coefficients in model cheese by fluorescence recovery after photobleaching (FRAP). *Food Chemistry, 133*(2), 551–556. http://dx.doi.org/10.1016/j.foodchem.2012.01.030.

Furukawa, R., Arauz-Lara, J. L., & Ware, B. R. (1991). Self-diffusion and probe diffusion in dilute and semidilute aqueous solutions of dextran. *Macromolecules, 24*(2), 599–605. http://dx.doi.org/10.1021/ma00002a039.

Gottwald, A., Creamer, L. K., Hubbard, P. L., & Callaghan, P. T. (2005). Diffusion, relaxation, and chemical exchange in casein gels: a nuclear magnetic resonance study. *The Journal of Chemical Physics, 122*(3), 34506. http://dx.doi.org/10.1063/1.1825383.

Gupta, A. K., & Gupta, M. (2005). Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. *Biomaterials, 26*(18), 3995–4021. http://dx.doi.org/10.1016/j.biomaterials.2004.10.012.

Hagman, J., Lorén, N., & Hermansson, A.-M. (2010). Effect of gelatin gelation kinetics on probe diffusion determined by FRAP and rheology. *Biomacromolecules, 11*(12), 3359–3366. http://dx.doi.org/10.1021/bm1008487.

Hagman, J., Lorén, N., & Hermansson, A.-M. (2012). Probe diffusion in κ-carrageenan gels determined by fluorescence recovery after photobleaching. *Food Hydrocolloids, 29*(1), 106–115. http://dx.doi.org/10.1016/j.foodhyd.2012.02.010.

Johansson, L., Skantze, U., & Loefroth, J. E. (1991). Diffusion and interaction in gels and solutions. 2. Experimental results on the obstruction effect. *Macromolecules, 24*(22), 6019–6023. http://dx.doi.org/10.1021/ma00022a018.

Johnson, E. M., Berk, D. A., Jain, R. K., & Deen, W. M. (1996). Hindered diffusion in agarose gels: test of effective medium model. *Biophysical Journal, 70*(2), 1017–1023. http://dx.doi.org/10.1016/S0006-3495(96)79645-5.

Jönsson, P., Jonsson, M. P., Tegenfeldt, J. O., & Höök, F. (2008). A method improving the accuracy of fluorescence recovery after photobleaching analysis. *Biophysical Journal, 95*(11), 5334–5348. http://dx.doi.org/10.1529/biophysj.108.134874.

Kohli, I., & Mukhopadhyay, A. (2012). Diffusion of nanoparticles in semidilute polymer solutions: effect of different length scales. *Macromolecules, 45*(15), 6143–6149. http://dx.doi.org/10.1021/ma301237r.

de Kort, D. W., van Duynhoven, J. P. M., Hoeben, F. J. M., Janssen, H. M., & Van As, H. (2014). NMR nanoparticle diffusometry in hydrogels: enhancing sensitivity and selectivity. *Analytical Chemistry, 86*, 9229–9235. http://dx.doi.org/10.1021/ac502211q.

Koynov, K., & Butt, H.-J. (2012). Fluorescence correlation spectroscopy in colloid and interface science. *Current Opinion in Colloid & Interface Science, 17*(6), 377–387. http://dx.doi.org/10.1016/j.cocis.2012.09.003.

Kvarnström, M., Westergård, A., Lorén, N., & Nydén, M. (2009). Brownian dynamics simulations in hydrogels using an adaptive time-stepping algorithm. *Physical Review E, 79*(1), 016102. http://dx.doi.org/10.1103/PhysRevE.79.016102.

Kwak, S., & Lafleur, M. (2003a). NMR self-diffusion of molecular and Macromolecular species in dextran solutions and gels. *Macromolecules, 36*(9), 3189–3195. http://dx.doi.org/10.1021/ma0213605.

Kwak, S., & Lafleur, M. (2003b). Self-diffusion of macromolecules and macroassemblies in curdlan gels as examined by PFG-SE NMR technique. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 221*(1–3), 231–242. http://dx.doi.org/10.1016/S0927-7757(03)00145-6.

Lafitte, G., Söderman, O., Thuresson, K., & Davies, J. (2007). PFG-NMR diffusometry: a tool for investigating the structure and dynamics of noncommercial purified pig gastric mucin in a wide range of concentrations. *Biopolymers, 86*(2), 165–175. http://dx.doi.org/10.1002/bip.20717.

Lafitte, G., Thuresson, K., Jarwoll, P., & Nydén, M. (2007). Transport properties and aggregation phenomena of polyoxyethylene sorbitane monooleate (polysorbate 80) in pig gastrointestinal mucin and mucus. *Langmuir, 23*(22), 10933–10939. http://dx.doi.org/10.1021/la701081s.

Langevin, D., & Rondelez, F. (1978). Sedimentation of large colloidal particles through semidilute polymer solutions. *Polymer, 19*(8), 875–882. http://dx.doi.org/10.1016/0032-3861(78)90191-X.

Lauffer, M. A. (1961). Theory of diffusion in gels. *Biophysical Journal, 1*(3), 205–213. http://dx.doi.org/10.1016/S0006-3495(61)86884-7.

Le Feunteun, S., & Mariette, F. (2007). Impact of casein gel microstructure on self-diffusion coefficient of molecular probes measured by 1H PFG-NMR. *Journal of Agricultural and Food Chemistry, 55*(26), 10764–10772. http://dx.doi.org/10.1021/jf071982v.

Le Feunteun, S., & Mariette, F. (2008a). Effects of acidification with and without rennet on a concentrated casein system: a kinetic NMR probe diffusion study. *Macromolecules, 41*(6), 2079–2086. http://dx.doi.org/10.1021/ma702248z.

Le Feunteun, S., & Mariette, F. (2008b). PFG–NMR techniques provide a new tool for continuous investigation of the evolution of the casein gel microstructure after renneting. *Macromolecules, 41*(6), 2071–2078. http://dx.doi.org/10.1021/ma702246m.

Le Feunteun, S., Ouethrani, M., & Mariette, F. (2012). The rennet coagulation mechanisms of a concentrated casein suspension as observed by PFG-NMR diffusion measurements. *Food Hydrocolloids, 27*(2), 456–463. http://dx.doi.org/10.1016/j.foodhyd.2011.09.008.

Lorén, N., Nydén, M., & Hermansson, A.-M. (2009). Determination of local diffusion properties in heterogeneous biomaterials. *Advances in Colloid and Interface Science, 150*(1), 5–15. http://dx.doi.org/10.1016/j.cis.2009.05.004.

Lorén, N., Shtykova, L., Kidman, S., Jarvoll, P., Nydén, M., & Hermansson, A.-M. (2009). Dendrimer diffusion in κ-carrageenan gel structures. *Biomacromolecules, 10*(2), 275–284. http://dx.doi.org/10.1021/bm801013x.

Lutti, A., & Callaghan, P. T. (2006). Measurement of diffusion in the presence of shear flow. *Journal of Magnetic Resonance, 180*(1), 83–92. http://dx.doi.org/10.1016/j.jmr.2006.01.009.

Mariette, F., Topgaard, D., Jönsson, B., & Söderman, O. (2002). 1H NMR diffusometry study of water in casein dispersions and gels.

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Journal of Agricultural and Food Chemistry, 50(15), 4295–4302. http://dx.doi.org/10.1021/jf0115948.

Masaro, L., & Zhu, X. (1999). Physical models of diffusion for polymer solutions, gels and solids. *Progress in Polymer Science, 24*(5), 731–775. http://dx.doi.org/10.1016/S0079-6700(99)00016-7.

Mason, T. G., & Weitz, D. A. (1995). Linear viscoelasticity of colloidal hard sphere suspensions near the glass transition. *Physical Review Letters, 75*(14), 2770–2773. http://dx.doi.org/10.1103/PhysRevLett.75.2770.

Moschakis, T. (2013). Microrheology and particle tracking in food gels and emulsions. *Current Opinion in Colloid & Interface Science, 18*(4), 311–323. http://dx.doi.org/10.1016/j.cocis.2013.04.011.

Mun, E. A., Hannell, C., Rogers, S. E., Hole, P., Williams, A. C., & Khutoryanskiy, V. V. (2014). On the role of specific interactions in the diffusion of nanoparticles in aqueous polymer solutions. *Langmuir, 30*(1), 308–317. http://dx.doi.org/10.1021/la4029035.

Netz, P. A., & Dorfmüller, T. (1995). Computer simulation studies of anomalous diffusion in gels: structural properties and probe-size dependence. *The Journal of Chemical Physics, 103*(20), 9074. http://dx.doi.org/10.1063/1.470018.

Nydén, M., & Söderman, O. (1998). An NMR self-diffusion investigation of aggregation phenomena in solutions of ethyl(hydroxyethyl)cellulose. *Macromolecules, 31*(15), 4990–5002. http://dx.doi.org/10.1021/ma971472.

Ogston, A. G. (1958). The spaces in a uniform random suspension of fibres. *Transactions of the Faraday Society, 54*(0), 1754–1757. http://dx.doi.org/10.1039/tf9585401754.

Olmsted, S. S., Padgett, J. L., Yudin, A. I., Whaley, K. J., Moench, T. R., & Cone, R. A. (2001). Diffusion of macromolecules and virus-like particles in human cervical mucus. *Biophysical Journal, 81*(4), 1930–1937. http://dx.doi.org/10.1016/S0006-3495(01)75844-4.

Perry, P. A., Fitzgerald, M. A., & Gilbert, R. G. (2006). Fluorescence recovery after photobleaching as a probe of diffusion in starch systems. *Biomacromolecules, 7*(2), 521–530. http://dx.doi.org/10.1021/bm0507711.

Petit, J. M., Roux, B., Zhu, X. X., & Macdonald, P. M. (1996). A new physical model for the diffusion of solvents and solute probes in polymer solutions. *Macromolecules, 29*(18), 6031–6036. http://dx.doi.org/10.1021/ma951159c.

Petrášek, Z., Ries, J., & Schwille, P. (2010). Scanning FCS for the characterization of protein dynamics in live cells. *Methods in Enzymology*, . http://dx.doi.org/10.1016/S0076-6879(10)72005-X.

Rathgeber, S., Beauvisage, H.-J., Chevreau, H., Willenbacher, N., & Oelschlaeger, C. (2009). Microrheology with fluorescence correlation spectroscopy. *Langmuir, 25*(11), 6368–6376. http://dx.doi.org/10.1021/la804170k.

Ross-Murphy, S. B. (1995). Structure-Property Relationships in Food Biopolymer Gels and Solutions. *Journal of Rheology, 39*(6), 1451–1463. http://dx.doi.org/10.1122/1.550610.

Rubinstein, M., & Colby, R. H. (2003). *Polymer physics*. Oxford University Press.

Salami, S., Rondeau-Mouro, C., Barhoum, M., van Duynhoven, J., & Mariette, F. (2014). Translational and rotational diffusion of flexible PEG and rigid dendrimer probes in sodium caseinate dispersions and acid gels. *Biopolymers, 101*(9), 959–965. http://dx.doi.org/10.1002/bip.22492.

Salami, S., Rondeau-Mouro, C., van Duynhoven, J., & Mariette, F. (2013a). PFG-NMR self-diffusion in casein dispersions: effects of probe size and protein aggregate size. *Food Hydrocolloids, 31*(2), 248–255. http://dx.doi.org/10.1016/j.foodhyd.2012.10.020.

Salami, S., Rondeau-Mouro, C., van Duynhoven, J., & Mariette, F. (2013b). Probe mobility in native phosphocaseinate suspensions and in a concentrated rennet gel: effects of probe flexibility and size. *Journal of Agricultural and Food Chemistry, 61*(24), 5870–5879. http://dx.doi.org/10.1021/jf304949c.

Sanders, L. M., & Hendren, R. W. (1997). *Protein delivery. Pharmaceutical biotechnology*.

Schuster, E., Hermansson, A.-M., Öhgren, C., Rudemo, M., & Lorén, N. (2014). Interactions and diffusion in fine-stranded β-lactoglobulin gels determined via FRAP and binding. *Biophysical Journal, 106*(1), 253–262. http://dx.doi.org/10.1016/j.bpj.2013.11.2959.

Schuster, B. S., Suk, J. S., Woodworth, G. F., & Hanes, J. (2013). Nanoparticle diffusion in respiratory mucus from humans without lung disease. *Biomaterials, 34*(13), 3439–3446. http://dx.doi.org/10.1016/j.biomaterials.2013.01.064.

Silva, J. V. C., Peixoto, P. D. S., Lortal, S., & Floury, J. (2013). Transport phenomena in a model cheese: the influence of the charge and shape of solutes on diffusion. *Journal of Dairy Science*, . http://dx.doi.org/10.3168/jds.2013-6552.

van der Sman, R. G. M., & van der Goot, A. J. (2009). The science of food structuring. *Soft Matter, 5*(3), 501–510. http://dx.doi.org/10.1039/b718952b.

Speedy, R. J. (1987). Diffusion in the hard sphere fluid. *Molecular Physics, 62*(2), 509–515. http://dx.doi.org/10.1080/00268978700102371.

Stylianopoulos, T., Diop-Frimpong, B., Munn, L. L., & Jain, R. K. (2010). Diffusion anisotropy in collagen gels and tumors: the effect of fiber network orientation. *Biophysical Journal, 99*(10), 3119–3128. http://dx.doi.org/10.1016/j.bpj.2010.08.065.

Takahashi, K., Kato, H., & Kinugasa, S. (2011). Development of a standard method for nanoparticle sizing by using the angular dependence of dynamic light scattering. *Analytical Sciences, 27*(7), 751–756. http://dx.doi.org/10.2116/analsci.27.751.

Tsai, D. S., & Strieder, W. (1986). Effective Conductivities of random fiber beds. *Chemical Engineering Communications, 40*(1–6), 207–218. http://dx.doi.org/10.1080/00986448608911698.

Valentine, M., Kaplan, P., Thota, D., Crocker, J., Gisler, T., Prud'homme, R., et al. (2001). Investigating the microenvironments of inhomogeneous soft materials with multiple particle tracking. *Physical Review E, 64*(6), 061506. http://dx.doi.org/10.1103/PhysRevE.64.061506.

Wang, B., Kuo, J., Bae, S. C., & Granick, S. (2012). When Brownian diffusion is not Gaussian. *Nature Materials, 11*(6), 481–485. http://dx.doi.org/10.1038/nmat3308.

Wassén, S., Bordes, R., Gebäck, T., Bernin, D., Schuster, E., Lorén, N., et al. (2014). Probe diffusion in phase-separated bicontinuous biopolymer gels. *Soft Matter*, . http://dx.doi.org/10.1039/C4SM01513D.

Weng, L., Liang, S., Zhang, L., Zhang, X., & Xu, J. (2005). Transport of glucose and poly(ethylene glycol)s in agarose gels studied by the refractive index method. *Macromolecules, 38*(12), 5236–5242. http://dx.doi.org/10.1021/ma047337w.

Wong, I. Y., Gardel, M. L., Reichman, D. R., Weeks, E. R., Valentine, M. T., Bausch, A. R., et al. (2004). Anomalous diffusion probes microstructure dynamics of entangled F-actin networks. *Physical Review Letters, 92*(17), 178101. http://dx.doi.org/10.1103/PhysRevLett.92.178101.

Zareh, S. K., DeSantis, M. C., Kessler, J. M., Li, J.-L., & Wang, Y. M. (2012). Single-image diffusion coefficient measurements of proteins in free solution. *Biophysical Journal, 102*(7), 1685–1691. http://dx.doi.org/10.1016/j.bpj.2012.02.030.

Please cite this article in press as: de Kort, D. W., et al., Nanoparticle diffusometry for quantitative assessment of submicron structure in food biopolymer networks: A review, Trends in Food Science & Technology (2014), http://dx.doi.org/10.1016/j.tifs.2014.11.003