5. Fluids and Soft Matter: From (Bio-) Molecules to Man

At the beginning of the previous chapter, dealing with channels and holes, we emphasized the relevance of these structures as a step in the evolution from an undifferentiated homogeneous mass to highly structured systems. Let us continue this evolution, eventually reaching the complexity of biological systems.

In our imagination, flourishing nature is associated with prosperous flowers and the lush green of meadows and trees. The special perspective on L'Aquila as given on page 319 doubtless provides such an impression. The first contribution to this chapter by Ed Cussler deals with transport barriers in films made from macromolecules, the key units of life. In contrast to polymer membranes, which are generally sought to ensure a high permeability, in the given case just the opposite is true. One strives rather to reduce the permeation flux of unwanted components such as radioactive cesium-137. Most interestingly, the way in which this was achieved in the given examples is not so different from a trick by which in the nearby village of Santo Stefano di Sessanio sheep have been prevented from freely diffusing into forbidden regions. The conference excursion takes us to this place, a village surrounded by lots of green as illustrated by the painting on page 320. It has a characteristic Medici tower, adumbrated in the painting. The tower symbolizes the excellent links of L'Aquila with Florence and the Medici family, stimulated by the joint interest in wool production. Thus the family emblem of the Medici is clearly visible on a number of buildings. A very interesting problem that they had to solve at that time was counting sheep. The solution that they adopted was to build a "sheep diffusion barrier". It limited the flux of the animals by allowing only one to pass at any given time. We are sure that these diffusion barriers act specific for sheep and will in no way impede your speed of propagation on exploring the most beautiful spots of Santo Stefano di Sessanio, following Taro Ito’s random walk as documented by the lovely paintings on pages 321 to 323.

The abstract on page 376, dedicated to the plenary lecture by Gero Vogl, deals with the propagation of isotopes (in material science) and of neobiota, the latter reminding us of the concepts used by Luca Cavalli-Sforza (page 139) for the exploration of social phenomena as re-settlement, immigration and emigration.

Farida Grinberg's contribution highlights the potential of NMR in the observation of reorientation phenomena in ordered macromolecular systems. Macromolecular ordering and the formation of membranes is the prerequisite for the compartmentation of water and solutes between different cells in a tissue and thus for life in general. Philip Kuchel's contribution deals with diffusion within and between a most vital representative of these compartments, the red blood cells. Extra- rather than intra-cellular space in our brain is in the focus of Charles Nicholson's story which concludes the diffusion papers of this book. The diffusion community will be proud to learn from it the important information on the role and functionality of space which becomes accessible by the application of diffusion concepts.
Diffusion Barriers

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1. Introduction

Diffusion is interesting because it is slow. As a result, many studies of diffusion seek ways to make the process fast and selective. For example, most membrane separations use thin polymer films to recover particular chemicals. Nitrogen is separated from air, and water is purified by ultrafiltration. This is an active and important area of diffusion research.

However, in other cases, we may seek to slow diffusion rather than to accelerate it. We may want protection from acids or oxygen. Paints and packaging are examples with this goal. In these cases we are seeking not to make diffusion fast and selective but to make it as slow as possible.

The obvious way to retard diffusion is to use thicker layers of less permeable polymers. However, as the data in Figure 1 show, this may often be difficult: a polymer which is highly impermeable to water may be highly permeable to oxygen. Even when we can achieve low permeabilities of many solutes, we almost always want still thinner coatings of still less permeable materials. In some cases, we want to reduce the permeability by a factor of 10,000. This is the goal for this research: how can we reduce the permeability of any diffusion barrier by a factor of 10,000?

In this paper, we discuss two possible routes for this reduction. The first is to incorporate immobile reactive groups into the diffusion barrier. These groups react with particular diffusing solutes and hence retard permeation of those solutes. Alternatively, we can use as diffusion barriers composite materials containing aligned impermeable flakes. The flakes force a tortuous path across the barrier. The first of these methods, reactive solutes, can reduce the unsteady state permeation by factor of 1000. The incorporation of aligned flakes can reduce both unsteady and steady state permeation by a factor of as much as 100. By combining these two methods, we can approach by our target of 10,000. The details of how this is accomplished are given in the remainder of this paper.
2. Slowing Diffusion with Reactive Barriers

To facilitate our discussion, we will focus on a particular experiment in a diffusion cell like that shown in Figure 2. This cell consists of two well-stirred volumes separated by a thin diffusion barrier. One of the volumes contains a high concentration of the solute of interest, but the other volume initially contains only solvent. By measuring the rate at which solute goes from the volume at high concentration to the volume which is initially solute free, we can evaluate the success of any diffusion barrier. While the apparatus in Figure 2 is specific to gases whose concentrations are measured by pressure or by gas chromatography, we can make a similar experiment for liquids where the concentration is measured by changes in pH or light absorption.
The key equation describing the concentration $c_1$ in the initially solvent-free volume is

$$\frac{c_1}{c_{10}} = \frac{PA}{V\ell} \left( t - \frac{\ell^2}{6D} \right)$$  \hspace{1cm} (1)$$

where $c_{10}$ is the high concentration in one volume, $P$ is the permeability of the diffusion barrier, $A$ is its cross-sectional area, $\ell$ is its thickness, $V$ is the volume of the originally solvent-free volume, $t$ is the time, and $D$ is the diffusion coefficient. Note that this equation predicts that after a lag, the concentration will rise linearly with time. This rise or leak rate is proportional to the permeability $P$ and is essentially a steady state value. The permeability is, of course, the product of a diffusion coefficient $D$ and a partition coefficient $H$, which is the equilibrium solubility in the barrier divided that in the adjacent solution. Thus, the leak rate depends on the permeability.

This linear region exists only after an initial time lag given by $\left(\ell^2/6D\right)$. This lag represents the time it takes for the solute to initially breach the diffusion barrier and approach its steady state leak rate. As Eq. 1 shows, the lag is not proportional to the permeability $P$, but to the diffusion coefficient $D$. Thus this basic experiment is
characterized by two quantities, a steady state leak rate proportional to the permeability and an unsteady state time lag proportional to the diffusion coefficient.

We now turn from this simplest reaction-free case to the case where there is a chemical reaction with a second immobile solute in the film. For simplicity, we consider only the case where the reaction is fast and irreversible, like an acid-base reaction. In this case, the concentration in the initially solute-free volume is given by

$$c(t) = \frac{c_0}{v\tau} \left( t - \frac{n\ell^2 c_0}{2Pc_{10}} \right)$$

The new parameters in this equation are $c_{20}$, the concentration of the immobile reactive species, and $v$, which is a stoichiometric coefficient. As before, this equation suggests that at larger times, the concentration will rise linearly with the time. The slope of this leak rate is again proportional to the permeability $P$ and is unchanged by the presence of the reagent. Thus the leak rate is the same in the nonreactive case and in the case of rapid irreversible chemical reaction.

Figure 3. Carbon Tetrachloride in Landfills

However, the lag in the reactive case is dramatically different. It still depends on the square of the membrane thickness $\ell$, but it now also depends on the concentration of the reagent species. Interestingly, it depends inversely not on the diffusion coefficient $D$, but on the permeability $P$. In practice, this new lag $(v\ell^2 c_0/2Pc_{10})$ is much larger than in the nonreactive case. As we will show below, it is often possible to make the lag 100 or 1000 times larger than in the nonreactive case. However, this better barrier exists only in unsteady state. Once the diffusion barrier is breached, it leaks just the way it did before.

We can illustrate these features by three examples. The first example is the escape of carbon tetrachloride from landfills lined with a barrier, which is sometimes called a “geotextile.” As Figure 3 shows, carbon tetrachloride quickly penetrates a thin
polyvinylalcohol barrier, as shown by the open circles. However, when nanoparticles of iron are added to this same membrane, the lag is increased dramatically by over 200 times, as shown by the filled circles. Similar results are obtained with other polymers like polyethylene, but the experiments are much, much longer. Thus, if the geotextile without iron is a good barrier for a month, it will be a good barrier for 15 years when nanoparticles of iron are blended into the polymer.

As a second example, we consider the containment of Cs-137, a radioactive isotope made in the manufacture of atomic weapons. This isotope is unusually dangerous because it is water soluble. It is currently stored in steel tanks which are nearing the end of their design life. If these tanks were to rupture, they could cause catastrophe in any communities which use water from the watershed around the radioactive storage.

![Figure 4. Cesium-137 Containment](image)

As shown in Figure 4, the cesium escape can be retarded by adding crystalline silicon titanate or “CST” to a polymer film which surrounds the radioactive storage. If there is no CST in the polymer film, this cesium gets across the film quickly. However, if the film contains 10 wt% CST, the cesium escape is retarded by a factor 80. Remember that this retardation is an unsteady state effect, and the cesium eventually does breach the film. While the rate at which it leaks is somewhat lower than the rate of the CST-free film, it does eventually leak. (We are not sure why this leak, once it occurs, is not the same rate as the leak in the absence of CST).

We can test this analysis more completely by looking at the simple system of zinc oxide incorporated into a polyvinylalcohol membrane and challenged by solutions of...
hydrochloric acid. In this case, we measure the lag as a change of pH. We then can compare this lag with the value calculated from Eq. 2. This comparison is given in Figure 5. The ordinate shows the measured lag time and the abscissa is its calculated value. The solid line, which is the theoretical result calculated from Eq. 2, has no adjustable parameters. The agreement between theory and experiment is good.

![Figure 5. Predicted Lag equals Experimental Lag](image)

We must again emphasize that the lag in these cases is an unsteady-state effect. While we can increase the lag by 1000 times or more, the barrier will eventually fail. The saving grace is that if our barrier previously worked for a week, the factor of 1000 means that our new barrier will work for almost 20 years.

3. Barriers with Aligned Flakes

We now turn to the case of thin polymer film containing aligned flakes of still less permeable material. One example which is convenient to study is a polymer like polyethylene filled with flakes of mica. In many cases, we will have a film which is still quite flexible. Thus we have the promise of having the mechanical properties of the original polymer but the diffusion properties of the crystalline, inorganic mica.

In this case, we are interested in how the permeability changes with the volume fraction of flakes \( \phi \) and the aspect ratio of the flakes \( \alpha \). This aspect ratio is defined as the intermediate dimension divided by the smallest dimension. Thus, for example, for ribbon-shaped flakes which are very long, the aspect ratio \( \alpha \) is the width divided by the thickness of the ribbon.

We are interested in two special cases, both of which involve dilute flakes, that is, cases where the volume fraction \( \phi \) is much less than one. In the first of these cases, often called the dilute limit, the product of concentration and shape \( \phi \alpha \) is also less than one. In this case, the permeability does not change much. In a second, more interesting case
called the semi-dilute limit, $\phi$ is much less than one but $\alpha \phi$ is much greater than one. In this case, the permeability of the barrier is dramatically changed.

How the permeability is changed in this semi-dilute case is best seen by referring to the same key experiment shown in Figure 2, and described for nonreactive films by Eq. 1, and for reactive films by Eq. 2. The corresponding result for flake-filled films is

$$c_i / c_{10} = \frac{PA}{vF} \left\{ \frac{1}{1 + \alpha^2 \phi^2} \left( t - \frac{\ell^2}{6D} \left[ \alpha^2 \phi^2 + 1 \right] \right) \right\}$$

(3)

This differs from Eq. 1 because of the factors in braces and square brackets. The factor in the braces gives the reduction in the leak rate caused by the presence of the flakes. The factor in the square brackets gives the change in the lag time caused by the flakes. Thus, unlike the case of chemically reactive barriers, the flakes both reduce the steady state leak rate and increase the unsteady lag.

![Figure 6. Permeability of Various Flake-Filled Films](image)

Some of the results obtained with membranes containing aligned flakes are shown in Figure 6. In this figure, the ordinate gives the permeability without flakes $P_0$ divided the permeability with flakes $P$. Large values of this ratio are evidence of effective barriers. The abscissa gives the factor in braces in Equation 3, which measures the steady-state effect of the aligned flakes. The experimental permeability shown on the ordinate correlates well with the function of flake shape and concentration shown on the abscissa. This is true for a wide variety of materials: mica in polyvinylalcohol, montmorillonite in...
polyethylene glycol, vermiculite in polyethylene glycol, silicate flakes in polyurethanes and polyamide flakes in low-density polyethylene. In every case, the correlation between theory and experiment is strong.

I must stress that the function in braces in Eq. 3 is controversial, challenged by many competing arguments. To expand on this controversy, we discuss two additional kinds of experiments. First, we made 100 µm flakes of titanium in a polydimethylsiloxane matrix using the techniques of photolithography. An example of these materials is shown at the right of Figure 7. At the left of Figure 7, we show that it takes three layers of square flakes to completely obscure the underlying surface. Thus we would expect that one layer of flakes would not cause a tortuous diffusion path, but rather would function primarily by solute being forced to neck down into a smaller area. Two layers would still leave gaps. Only three layers will begin to cause tortuosity.

![Figure 7. Lithographically-Made Flakes](image)

These results are supported by the gas permeation experiments in an apparatus like that in Figure 2 and shown in Figure 8. On the ordinate of this graph is the concentration difference of helium divided by the initial concentration. These concentrations are measured as partial pressure differences. On the abscissa is the time divided by the membrane thickness. Putting in one layer of flakes retards the diffusion; putting in three or five layers retards it by more but by the same amount. This is consistent with Eq. 3.
As a second example, we show the permeability across a block copolymer of polystyrene (PS) and polylactic acid (PLA). Polystyrene is a glass and polylactic acid is a rubber. Under most circumstances, the permeability would be dominated by that of the more permeable polymer, that is, by the polylactic acid. In this particular case, however, the block copolymer is chosen so that it self-assembles into a layered structure rather like the structure of the mica flakes. If we measure the helium concentration as a function of time, we obtain different permeabilities \( B \), in the rubber \( P_{PLA} \), the glass \( P_{s} \), and the composite \( P \), as shown in Figure 9. The data show that the rubber is highly permeable, and that the glass is considerably less permeable. The composite is intermediate but is consistent with a permeability calculated as resistances in series:

\[
\frac{1}{P} = \frac{1 - \phi}{P_{PLA}} + \frac{\phi}{P_{PS}}
\]

Again, we have a permeability of a composite structure which is in agreement with what we would expect from diffusion theory.
4. Barriers with Reactions and Flakes

Finally, we might ask whether the effects of reaction, which produce an unsteady state result of a factor of 1000 or more, can be combined with the presence of flakes, which produce steady state permeability changes up to a factor of 50. That these two effects can be combined is shown by the data in Figure 10. The ordinate gives the measured experimental lag, and the abscissa reports the predicted lag. We see good agreement for systems that contain mica, zinc oxide challenged by hydrochloric acid, and a combination of mica and zinc oxide. Thus we believe that these two effects can be effectively combined. We should stress, however, that this is true only for the unsteady state case where both reactions and flakes are effective. In the steady state case, we would expect the reactions to have relatively little effect.
5. Conclusions

In this paper, we present a strategy for improving the barrier properties of any polymer. We can use reactive membranes to increase the lag time by a factor of 1000. We can use flake-filled membranes to increase the lag time by a factor of 50 and reduce the steady-state leak rate by a similar factor. We can even get orientation from self-assembly of block copolymers, although the permeation may be significantly faster than what can be expected with inorganic flakes. In any case, we can get close to our original objective of reducing the permeability of any material by a factor of 10,000.

Further Reading


Reorientations Mediated by Translational Displacements in Confined Liquid Crystals Studied by Field Cycling NMR Relaxometry and Monte Carlo Simulations

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Abstract
Ordering effects and low-frequency molecular dynamics in the nematic liquid crystals confined in mesoscopic pores was studied with the help of field cycling (FC) Nuclear Magnetic Resonance (NMR) relaxometry and Monte Carlo simulations. Proton relaxation rates were measured above the bulk isotropisation temperature in the broad frequency range between 2 kHz and 7 MHz. The average pore radii of confinements were between 1.5 and 15 nanometers. The relaxation dispersion curves in the confined materials exhibited strong deviations from the behaviour in bulk. In a few kHz range, a dramatic enhancement of the relaxation rates exceeding two orders of magnitude compared to the bulk sample was observed. The low-frequency value of the relaxation rate exhibited a strong dependence on the pore size. Experimental findings were interpreted in terms of the surface induced orientational order and diffusion between the sites with different orientations of local directors. The analysis was supported by Monte Carlo simulations of the reorientations mediated by translational displacements (RMTD) in spherical cavities.

1. Introduction
In recent years, molecular ordering of soft systems on the meso- and macroscopic length scales has been successfully exploited in molecular engineering and in production of new materials with predefined properties. Molecular ordering is in turn accompanied by multiple molecular (individual or collective) dynamic processes that tend to range over many time decades. While structure properties of these materials are rapidly getting more and more accessible due to advances in optical and scattering techniques, one of the challenging related problems refers to understanding of their complex dynamic features at the molecular level and addressing the relationship between structure, dynamics and function with a predictable power.

Specific functionalities of new materials often exploit self-assembling and molecular ordering properties on the meso- and macroscopic length scales. This also refers to liquid crystals confined in nano-structured materials used in electro-optical applications and in sensors. In particular, liquid crystals constrained in micro- or nanoscaled environments
with solid interfaces, tend to exhibit partial orientational order \([1, 2]\) induced by surface interactions. This type of ordering can extend over mesoscopic length scales and persists well above the bulk isotropisation temperature, \(T_{NI}\).

NMR has proven to provide powerful tools for studying confined liquid crystals \([3]\). Especially informative in this context are the methods permitting one to access molecular dynamics in the low-frequency limit down to a few kHz. In soft materials, the low frequency scale is characteristic of the unique mechanisms of (collective or individual) molecular motions that are not observed in liquids of low viscosity. The prominent examples are given by the so-called Orientational Director Fluctuations in nematics or long back-bone reptation-like motions of macromolecular chains. Another example is given by the so-called RMTD mechanism observed in polar liquids \([4, 5]\) and liquid crystals confined in mesoscopic pores. In the case of polar liquids under strong adsorption conditions, diffusive displacements along the surface are described with non-Gaussian propagators and are characteristic of the super-diffusive time dependence of the mean-square displacement values. In the case of liquid crystals, molecular ordering occurring at the interfaces gives rise to the long-lived correlations in re-orientational dynamics of molecules.

One of the most powerful NMR techniques which permits one to probe low frequency dynamics in the range covering several orders of magnitude is given by the field cycling relaxometry \([4]\). The natural extension of the FC relaxometry to even longer time scales is provided by the technique known as the Dipolar Correlation Effect on the stimulated echo \([6, 7]\). Also, the long time scale translational molecular dynamics can efficiently be probed in diffusion studies using strong pulsed magnetic field gradients \([8, 9]\). Worth noting is, however, that the surface-layer molecules in confined systems are not easily accessible in direct diffusion studies because typically their relative fraction in the whole sample tends to be too small for a direct detection.

In this contribution, I am going to examine ordering effects in confined liquid crystals with the help of FC NMR relaxometry and Monte Carlo computer simulations. Confining materials were porous glasses with pore sizes in the mesoscopic range where the surface constraints are the strongest.

2. Materials and methods

Samples studied were Controlled Porous Glasses (CPG) and Bioran Glasses filled with nematic liquid crystal 4′-n-pentyl-4-cyanobiphenyl (5CB). \(T_{NI}\) of 5CB is 308.5 K.

The mean pore radii of the CPG samples denoted as CPG-1.5 and CPG-4 were 1.5 nm and 4 nm, respectively. The mean pore radii of the Bioran samples denoted as Bioran-5 and Bioran-15 were 5 nm and 15 nm, respectively. Frequency dependences of the spin-lattice relaxation rates, \(T_1^{-1}\), were measured at 323 K with the help of a home-built FC relaxometer at the University of Ulm, Germany.

3. Monte Carlo Simulations

Simulated dipolar correlation functions are defined as

\[
G^\text{red}_i(t) = \frac{\langle Y_{i,\hat{z},0} Y_{i,\hat{z},t} \rangle}{\langle Y_{i,\hat{z},0}^2 \rangle},
\]  

(1)
where $Y_{2,k}$ ($k=0,1,2$) are second order spherical harmonics describing the instantaneous orientation of the internuclear vector relative to the external magnetic field. Random-walk Monte Carlo simulations were performed for a spherical cavity of a given radius, $R$. In each cycle time, $\Delta t << t$, a random step of a fixed length, $\Delta l << R$, was generated and $G_{k}^{\text{red}}(t)$ was evaluated as described in References [10, 11]. All relevant time and length scales are expressed in terms of the predefined values of $\Delta l$ and the diffusion coefficient, $D$. The (predefined) value of the diffusion coefficient was equal to $10^{-10}$ m$^2$s$^{-1}$, the step length was set to 0.2 nm.

The simulated correlation functions were used to evaluate the laboratory frame spin-lattice relaxation rates [12]:

$$\frac{1}{T_1(\omega_0)} = K[I_1(\omega_0) + 4I_2(2\omega_0)],$$

where $\omega_0$ is the Larmor frequency, $K = \frac{3}{20}\gamma^4\hbar^2r^2\langle \frac{\mu_0}{4\pi} \rangle^2$, $\gamma$ is the gyromagnetic ratio of the nuclei, $\hbar$ is the Planck constant divided by $2\pi$, $r$ is the internuclear distance, and

$$I_1(\omega) = \int G_1(t)\exp(i\omega t)dt.$$ (3)

4. RMTD and the exchange model

Effects of surface ordering on nuclear magnetic relaxation were analysed for a simple spherical geometry under consideration of the two-phase exchange and RMTD processes. Within the sphere, two areas are distinguished: a) the isotropic bulk-like area inside the sphere and b) the surface-ordered layer of thickness $\delta r$. Generally, the total correlation function $G_k^{\text{red}}(t)$ can be decomposed in four partial correlation functions [10] related to the two fractions of molecules initially and finally in the same phase (surface layer or bulk) and the two fractions of molecules initially and finally in different phases. Any correlations occurring in the bulk-like area were not considered, anticipating that molecular reorientations in this phase are isotropic and fast and thus do not contribute to the observed relaxation. In practice, this was realized by replacing the spherical harmonics by zero any time when a spatial position of the molecule was in the bulk-like area. Within the surface-ordered layer, the current value of $Y_{2,k}$ was determined by the preferential orientation (in this case, perpendicular) relative to the surface at the instantaneous position of the random walker. Under these conditions, $G_k^{\text{red}}(t)$ reduces to [10]:

$$G_k^{\text{red}}(t) = f_{s,s}\langle Y_{2,k}(0)Y_{2,k}(t)\rangle_{\text{RMTD}},$$

where $f_{s,s}$ is the fraction of molecules being initially and finally in the surface layer. The abbreviation RMTD stands for the mechanism [4, 5] describing molecular reorientations due to diffusion between the surface sites with different preferred molecular orientations (different directors of the surface order). Between times 0 and $t$, the correlation to the initial molecular orientation in the surface layer may temporarily be lost, as molecules
perform extended excursions to the bulk-like area, but it tends to restore at much longer times, as molecules repeatedly return to the ordered layer and adopt the preferential orientations again. The correlation function for the RMTD process thus depends on such factors as the surface topology, geometry of the pore space, molecular diffusivity and interactions with the surface.

The fraction \( f_{s,s} \) is generally a function of time. At very short times, for which the probability of the exchange between the ordered and the bulk-like populations tends to zero, \( f_{s,s} \approx f_s \), where \( f_s \) is the (constant) population of the surface layer. At long times, for which the initial and final probabilities to be in the surface layer become independent of each other, \( f_{s,s}(t) \approx f_s^2 \). The function \( f_{s,s}(t) \) in Eq. (4) thus decays from the initial value \( f_s \) to the value \( f_s^2 \) and resembles an exchange-loss process, that is, the loss of molecules populating the surface phase at time 0 but being rather in the bulk-like phase at time \( t \).

3. Results and Discussion

Figure 1 shows typical shapes of the reduced correlation functions, Eq. (1), simulated for a sphere of \( R = 50 \) nm and different values of \( \delta r \), curves 1-5. The correlation functions
clearly exhibit two components with different characteristic decay rates. The initial fast loss of correlations \((t < 10^{-6} \text{ s})\) is due to the exchange losses represented by the factor \(f_{ss}(t)\). The long-time tails of the correlation functions are not affected by the exchange losses and are governed entirely by the RMTD process, that is, they are controlled by orientations adopted by the random walker in the surface ordered layer.

Two components of the correlation function can be distinguished by repeating the simulation with the RMTD mechanism inactivated, i.e., assuming a constant fixed molecular orientation at any position of the surface layer. In this case, the simulation results, Eq. (4), represent merely the function \(f_{ss}(t)\). The latter is demonstrated in Fig. 1 by curve 6 for \(\delta r\) equal to 3 nm. The crossover from the initial value to the constant value in the long-time limit is clearly distinguishable and gives an estimate of the mean exchange time between two phases. For the simulation conditions used in Fig. 1, the mean exchange time is of the order of \(10^{-6} \text{ s}\).

![Figure 2](image-url)  

Figure 2. Normalised frequency dependencies of the spin-lattice relaxation rates, curves 1-7, evaluated from the simulated correlation functions. The curve parameter refers to different values of \(\delta r\) and \(R\). The experimental data points refer to 5CB in CPG-1.5 and CPG-4 at 323 K.

The long-time tails representing the correlation function of the RMTD mechanism, \(\langle Y_{kk}(0)Y_{kk}(t)\rangle_{\text{RMTD}}\), can be well fitted by an exponential function, \(C \exp(-t/\tau_{\text{RMTD}})\), where the time constant, \(\tau_{\text{RMTD}}\), was fitted to \(2.4 \pm 0.24 \times 10^{-4} \text{ s}\). The fits are shown as the straight lines in Fig. 1. The values of the pre-factor \(C\) depend on the thickness of the oriented layer (or on the population of the ordered molecules) as shown in the inset of Fig. 1.

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Figure 2 shows the simulated frequency dependences of $T_1^{-1}$ normalized by the low-frequency values, curves 1-7. Simulation data are shown for various thicknesses $\delta r$ within a cavity of the same size ($R = 30$ nm), curves 3-6, and for the cavities of different sizes ($R = 60$ nm, 50 nm, 20 nm), curves 1, 2 and 7. Figure 2 shows that, given the same cavity size, the width of the surface-ordered layer strongly affects the dispersion slopes, compare curves 1-4, but not the cut-off frequency of the dispersion (the cross-over between the plateau and the power-law dependence). As demonstrated in Fig. 2, the

![Graph showing frequency dependences of $T_1^{-1}$ for different thicknesses and cavity sizes.](image)

Figure 3. Experimental frequency dependencies of the spin-lattice relaxation rates of bulk 5CB and 5CB confined in CPG-1 and CPG-4 and in Bioran-5 and Bioran-15.

slopes "span" the power laws between $v_0^{-2}$ and $v_0^{-0.5}$ represented graphically by the straight lines. Thinner layers are characteristic of flatter slopes as a result of the increasing relative fraction of the bulk-like phase. Increasing the pore radius shifts the cut-off frequency towards lower values but does not considerably affect the slope, provided that the thickness of the ordered layer remains in the same proportion to the pore size.

Experimental relaxation rates of 5CB confined in CPG and Bioran glasses are shown as a function of the Larmor frequency in Fig. 3 in the range between 2 kHz and 7 MHz. The measuring temperature was 323 K well above $T_{NI}$. A strong impact of confinements on the low-frequency behavior of the relaxation rates was observed. That means, in contrast to bulk 5CB where the relaxation rates are practically independent of
the frequency (as typically expected for ordinary liquids below the megahertz range), the confined samples exhibit a pronounced dispersion. At the lowest frequency limit, the values of $T_1^{-1}$ were the highest for the sample with the smallest pore size (1.5 nm) and exceeded that of bulk 5CB by more than two orders of magnitude. Increasing the pore size resulted in a dramatic decrease of the relaxation rates.

Figure 4. Normalised values of square root longitudinal ($K_1 \times 0.5$) and transverse ($K_2 \times 0.5$) relaxation rates of 5CB in pores as a function of the pore radius.

Strong enhancement of the relaxation rates in the kHz range in the confined samples can be attributed to the orientational ordering at the surface and the RMTD mechanism. The orientational anisotropy stabilized by solid interfaces prevents a complete averaging of dipolar interactions by molecular motions. Diffusion between the regions with different surface orientations and different levels of orientational anisotropy then produces a strong relaxation mechanism in the kilohertz frequency range. This mechanism is also relevant for the transverse relaxation [11, 13].

The Landau-de Gennes theory predicts that the order parameter decreases exponentially with increasing distance, $r$, from the wall as

$$S(r) \propto \exp \left( -\frac{r}{\xi} \right)$$  \hspace{1cm} (5)
where $S$ is a local order parameter, $\xi$ is the characteristic decay constant called the nematic correlation length. The relaxation rates are generally proportional to the square of the (residual) dipolar couplings. As shown in the Reference [2], in confined liquid crystals above $T_{NI}$ the square roots of the relaxation rates then appear to be proportional to $S$.

The nematic correlation length in the investigated materials was roughly estimated in Reference [13] as approximately 3 nm at 313 K. In CPG-1.5, therefore, the dominating fraction of confined molecules should exhibit a relatively strong ordering. In samples with larger pores, that is, in CPG-4 and Bioran glasses, the interior middle area is essentially less ordered, and the relative fraction of the more “bulk-like” fraction increases for bigger pores. Consequently, the decrease of the relaxation rates with increasing the pore size is due to the averaging effect of translational molecular diffusion between the regions with different degrees of local anisotropies. This finding complies with the earlier reported measurements of the transverse relaxation and cross-relaxation rates [13, 14] in the CPG samples.

Figure 4 shows the pore size dependence of the square roots of the longitudinal relaxation rates in the low frequency limit (at approximately 10 kHz) in comparison to that of the transverse relaxation rates. The latter were earlier used for the estimation of the nematic correlation length [11]. The similarity in the behavior of both relaxation rates as a function of the pore size allows one to conclude that transverse and longitudinal relaxation at low frequencies are governed by the same mechanisms and that the field cycling relaxometry is well suited for the quantitative study of interfacial ordering occurring in confined liquid crystals.

The normalized dispersion curves of the CPG samples at 323 K are shown once again in Fig. 2 together with the simulated data. The low frequency value of $T_1^{-1}$ of the bulk sample was subtracted from the measured values of the relaxation rates of the confined samples in order to account for the contribution of fast bulk-like motions (not taken into account in the simulations). The experimental dispersions cannot be described as a Lorentzian type mechanism (slope $-2$) but rather exhibit power law frequency dependences with slopes between $-1$ and $-0.5$. This correlates with the results reported in the Reference [15] for 5CB confined in much bigger, compared to our samples, pores of 72 nm. As shown in Reference [15], the RMTD mechanism gives rise to the inverse square root frequency dispersion.

The cut-off frequency observed for the CPG glasses corresponds to much bigger radii used in the simulated data than their actual pore sizes. This is, however, not surprising since diffusion in our samples is not restricted to a single cavity. During the typical observation time, the molecules probe much larger distances (exceeding 100 nm) of the random pore space compared to the pore radii. The cut-off frequency, however, permits one to get an estimate of the effective curvature (or the smallest cut-off wave number) probed by diffusing molecules which is of the order of a few tenths of nanometers [14].

**Acknowledgments.** I thank Prof. Dr. R. Kimmich for a fruitful co-operation during my multi-year stay in his laboratory at the University of Ulm. The experimental part of this work was done there. I further thank Prof. Dr. J. Kärger for the excellent research conditions in his laboratory at the University of Leipzig.
References


NMR Diffusion Diffraction and Diffusion Interference from Cells

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Abstract

Pulsed field gradient spin-echo (PGSE) NMR spectroscopy is the definitive means for measuring translational motion of molecules in free solution and in heterogeneous systems. A unique ‘twist’ on the method is that in some systems in which diffusion is restricted the PGSE experiment yields information on the geometrical properties of the confining boundaries. When applied to red blood cells (RBCs) in suspensions, using intense magnetic field gradients (around 10 T m⁻¹), the graph of normalized NMR-signal intensity versus the magnitude of the field gradients has the form of the diffraction and interference patterns that are seen in physical optics. We review here the nature of these so-called q-space plots and discuss a data-processing method that adds objectivity to estimates of the mean RBC diameter. Convection potentially interferes with the veracity of these measurements so an experiment is reported in which a cell-free sample was deliberately made to flow. The very simple analysis of flow diffraction yielded estimates of flow that were in remarkable agreement with gravimetric measurements. Finally, in a theoretical study using a model of uniformly arrayed octagonal prisms that were ‘morphed’ in a systematic way, the dependence of the form of q-space plots on prism shape and packing density was obtained. This showed that elaborately shaped q-space plots can be obtained from simple periodic arrays of ‘cells’. The uniqueness or otherwise of shapes of q-space plots, and the prospect of generally solving the inverse problem whereby q-space analysis yields detailed information on packing arrangements is poised for further detailed investigations.

Keywords: convection; diffusion diffraction; diffusion interference; erythrocyte; flow diffraction; physical optics; q-space plot; pulsed field gradient spin-echo; red blood cell; restricted diffusion

Abbreviations: Ht, haematocrits; PGSE, pulsed field gradient spin-echo; RBC, red blood cell; SGP, short gradient pulse.

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1. Introduction

The thermally driven random motion of water and solute molecules in cells is a major factor in determining the access of substrates to enzymes that act upon them and hence carry out metabolic processes [1]. Pulsed field gradient spin-echo (PGSE) NMR [2,3] has yielded estimates of the diffusion coefficients in heterogeneous systems using ‘classical’ methods of data analysis [4,5] that continue to be relevant with modern NMR pulse sequences. The mobility of water [6], low molecular weight solutes including metabolites [7,8], and proteins such as haemoglobin inside the human red blood cell (RBC) [9], have been measured this way. From a knowledge of the apparent diffusion coefficients the microviscosity and mean residence times in tissue compartments and cells have been estimated [10].

Compartmentation of water and solutes between different cells in a tissue, and between organelles within a single cell, is one means whereby metabolic processes are regulated. Changes in cell shape occur naturally and yet the kinetics and energetics of the processes are poorly understood. Such shape changes are exemplified by the autonomous transition of the human RBC from its usual biconcave-disc shape, to an ‘early’ echinocyte (Greek, echinos = sea urchin) that has ~20 rounded projections, then to a ‘late’ echinocyte that has sharp membranous spikes, finally to a spherocyte, that has few membranous protrusions, with a total volume that is ~75% of the original value. Under the appropriate conditions of zero glucose concentration, the first shape transition takes ~5 min, while the later stages evolve over 20 h [e.g., 11,12]. Experimental delineation of cell shape in concentrated suspensions of RBCs such as those encountered in the blood are not available by conventional light microscopy; but PGSE NMR can uniquely provide estimates of cell dimensions by exploiting signals from molecules that undergo restricted diffusion [13-16]. It is this aspect of PGSE NMR that we focus on in this paper via the following four related topics.

2. q-Space of RBCs

For a solute in an isotropic medium undergoing unbounded diffusion a plot of the natural logarithm of the PGSE NMR signal intensity versus the square of the amplitude of the magnetic field gradients is a straight line. The negative slope of this so called Stejskal-Tanner plot is proportional to the diffusion coefficient of the solute [2,3]. Or, a plot of the intensity versus $g$ (not $g^2$) is a smooth monotonically-strictly-decreasing half-

![Fig. 1. $^1$H NMR q-space plot derived from the $^1$H$_2$O signal at 400 MHz from water in a ~1 mL suspension of human RBCs (haematocrit, volume fraction that was cells ~70%) at 25°C in a 10-mm NMR tube. $E[q, \Delta]$ is the spin-echo PGSE NMR signal intensity relative to the value obtained when the magnetic field gradients, $g = 0$; the gradients were in the direction of the main magnetic field $B_0$ and had a maximum value of 9.4 T m$^{-1}$, $g = (1/2\pi) \gamma g \delta$, where $\gamma$ is the magnetogyric ratio; and $\delta$ is the pulse duration in the PGSE experiment. Annotation highlights key features. (Adapted from [16].)
Gaussian shape. On the other hand if diffusion is restricted in the sample the latter curve is no longer simple. In an extreme case, with a highly ordered sample the outcome is like that seen in Fig. 1; this shows a typical $q$-space plot obtained from the $^1$H$_2$O signal from a suspension of human RBCs.

The main features evident in Fig. 1 are manifestations of what is called $q$-space diffusion-diffraction [2] but there is an additional minor but very reproducible feature [11,12,16,17] from what is called diffusion interference; it is so named because it arises from diffusion between the RBCs and is similar to the situation seen with two- or multi-slit interference in physical optics [2, 18]. The diffraction effect arises from water that diffuses in a constricted way inside the cells and consequently produces an effect like that for diffraction of light through a single slit [18], i.e., a signal of squared sinc-function shape (see below).

It is worth emphasizing that while $q$-space plots have been reported from other samples, both biological and inanimate ones, none to our knowledge show the same high level of structural features with up to four maxima in the curves. This favourable outcome is ascribed to the precise size discrimination that occurs in the production of RBCs in the mammalian bone marrow; thus the cells as particles have a very narrow size distribution. Furthermore, the biconcave-discs become aligned in the magnetic field of the NMR spectrometer, $B_0$, with their flattish faces parallel to it thus constituting a very ordered system [17,19].

The mechanism of the alignment does not reside with paramagnetic haemoglobin, as is often supposed; it even occurs when the cells are saturated with oxygen or carbon monoxide that renders the iron in the heme of haemoglobin low-spin FeII and diamagnetic. The effect is due to the diamagnetic anisotropy of the phospholipids in the RBC plasma membranes for which the minimum-energy orientation is perpendicular to $B_0$; the maximum number of phospholipid-fatty acid chains are aligned at right angles to $B_0$ when the disc-planes of the RBCs are parallel to $B_0$. Thus, $q$-space data can be used to estimate the mean diameter of the RBCs in the sample [17].

For cylinders lying across the direction of the field-gradient the mean cell diameter is given by $1.22/ q_{\text{min}}$, where $q_{\text{min}}$ is the value of $q$ at the first diffraction minimum of the plot [17]. Although the human RBC has the shape of a biconcave disc, it is closely approximated by a short cylinder of main diameter ~6-8 μm.

3. Feature Enhancement

Kärger et al. [20,21] proposed the idea, and then in studies of yeast cells, Cory and Garroway [13] emphasized that an inverse Fourier relationship exists between $q$ and the mean displacement, $\tau$, of the molecules moving during the ‘diffusion time’ $\Delta$ of the PGSE experiment (see Eq. 6 below). Thus Fourier transformation of the spin-echo attenuation in a $q$-space plot, like that in Fig.1, should yield a curve that represents the distribution of dynamic displacements that arise from water diffusing in the restricted spaces of the RBC suspension. This distribution of $\tau$ values is called the average propagator (see Eq. 3 below). While it is possible to directly apply a numerical Fourier transform to the data it turns out to be useful to enhance the usually featureless $q$-space plot; to this end we introduced a numerical procedure that is based on applying numerical
filtering and feature-enhancement via a Mathematica [23] program: (1) The 16-92 points \( N \) in a \( q \)-space data set \( (q_i, s_i) \) are interpolated with a shifting cubic-spline using the standard Mathematica function, \textbf{Interpolation}; (2) the interpolation function which is a smooth function of as many points as required (certainly many more than the original data set) is numerically differentiated, twice using the function \textbf{D}; (3) a Blackman-Harris bell-shaped filter \((\sin^4(\pi q_i/q_N))\) is then applied; and (4) then the data are numerically Fourier transformed by using the standard Mathematica function \textbf{Fourier}. With due attention given to the physical scales in the graphs, the signal for \(^1\text{H}_2\text{O}\) in human RBCs yields a spectrum of mean displacements of the diffusing water [22]. A typical example is shown in Fig. 2.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{fig2}
\caption{PGSE NMR \( q \)-space plots of water diffusing in suspensions of human RBCs of three different packing densities (haematocrits; \( \text{Ht} \)). \( A \), The logarithm of the signal attenuations versus \( q \). \( B \), The second derivatives of the data in \( A \), that had been numerically interpolated with a shifting cubic-spline into the non-logarithmic version of the data. \( C \), The data in \( B \) were multiplied by a Blackman-Harris filter and then Fourier transformed. The largest peak had an ordinate value that corresponded to a mean dynamic displacement of \( 6.2 \mu m \). \( \text{Ht} = 58\% \) for upper row of dots and dashed lines in \( B \) and \( C \); \( \text{Ht} = 48\% \) for the central row of dots and the thin solid lines in \( B \) and \( C \); and \( \text{Ht} = 40.8\% \) for the bottom row of dots and thick solid lines in \( B \) and \( C \). (Adapted from [22].)\end{figure}

Figure 2C shows that the main diffusion-restricting barriers (the cell membranes) have a spacing of \( 6.2 \mu m \) between them within a single cell; this is less than the known main diameter of \( 8 \mu m \) of the human RBC. The lower estimate arises because water-exchange across the membrane is rapid which decreases the apparent diameter of the cell; also each cell has a circular and not a rectangular cross-section [16].

The ‘signatures’ (special features) in \( q \)-space plots that are brought about by canonical forms of packing of biconcave RBCs has been extensively studied by using Monte Carlo computer simulations of restricted diffusion [24-26]. We conclude that, given the natural variations in RBC size and shape, the secondary features of \( q \)-space plots evident in these simulations, while distinct for ideal packing arrays, are unlikely to be interpreted...
unambiguously in real samples. On the other hand, for highly uniform geological samples, this may be possible.

Monte Carlo methods are very powerful for simulating diffusion in systems of almost any conceivable arrangement of restrictions and obstructions [12,24-26]. However, it is still important to gain impressions of the shapes of \( q \)-space plots that would be obtained from canonical systems of diffusion-confining arrays for which there are analytical solutions to the relevant NMR-diffusion equations. We consider this matter next.

4. Fundamental \( q \)-Space Theory

The following theory explains how, with conventional PGSE NMR \( q \)-space experiments, the coherence features in the plots are simply related to the geometry of the diffusion-confining bodies in the sample [20,21,27]. The theory for spins that diffuse in the space(s) outside geometrically well-defined bodies is much less well developed and to date such analyses on cellular systems tend to have exploited Monte Carlo simulations [e.g., 11,16,24-26].

The theoretical treatment of restricted diffusion in PGSE NMR experiments [2,3] that yields most insights is the one in which the duration, \( \delta \), of the field gradient pulses is taken to be so short that spins do not move significantly during the pulses; this is called the short gradient pulse (SGP) approximation. In this situation the normalized spin-echo signal intensity \( E[q, \Delta] \) (in which the effects of relaxation during the experiment are cancelled out by normalizing the signal intensities to the one for which \( g = 0 \)) is described by the expression:

\[
E[q, \Delta] = \rho[r_0 | r, \Delta] \exp[i \delta g \cdot (r - r_0)] d\nu d\nu
\]  

where \( \rho[r_0 | r, \Delta] \) is the conditional probability density that describes the probability of a spin starting at the position \( r_0 \) and ending at \( r \) in the time interval \( \Delta \); \( \Delta \) is the time interval between the field gradient pulses of amplitude \( g \) in a specified direction hence it is a vector \( g \); and the vector \( q = (2 \pi)^{-1/2} \delta g \) describes a metric space that is the reciprocal of the space of displacement vectors \( (r - r_0) \). To highlight this idea we write Eq. (1) with \( R = (r - r_0) \) to give,

\[
E[q, \Delta] = \int \rho[r_0 | r, \Delta] P[r_0 + R, \Delta] \exp[i 2 \pi q \cdot R] d\nu d\nu
\]  

In the development of the whole area of SGP-\( q \)-space theory the ‘key insight’ was that of the average propagator [20,21]; it is the ensemble average probability density of the displacement vector \( R \) which is defined by,

\[
\overline{P}[R, \Delta] = \int \rho[r_0 | r_0 + R, \Delta] d\nu
\]  

Hence by substituting this expression into Eq (2) we obtain,
\[ E[q, \Delta] = \int \tilde{P}[R, \Delta] \exp[i 2 \pi q \cdot R] dR \]

Equation (4) is the definition of the Fourier transform of \( \tilde{P}[R, \Delta] \) where the variables in the conjugate spaces are \( q \) and \( R \). Thus,

\[ \tilde{P}[R, \Delta] = \int E[q, \Delta] \exp[-i 2 \pi q \cdot R] dq \]

When the time interval between the gradient pulses, \( \Delta \), is so large that each spin in a confined region ‘maps out’ the full extent of a restricting region, then its final position is independent of its starting position. Thus the sole determinant of the final position of each spin is the positional probability density \( \rho[r] \) of the compartment itself and it is not related to the value of the diffusion coefficient at all; thus Eq. (1) becomes:

\[ E[q, \infty] = \int \rho[r_0] \exp[i 2 \pi q \cdot r_0] dr_0 \int \rho[r] \exp[-i 2 \pi q \cdot r] dr \]

\[ E[q, \infty] = S^*[q] S[q] = |S[q]|^2 \]

where \( S[q] \) is the counterpart of the conventional k-space NMR image expression [27]. The probability density \( \rho[r] \), for the situation in which there is a gradient only in the \( z \)-direction is merely the projection of the shape of the confining cavities onto the \( z \)-axis. Hence the approach to the mathematical analysis that is required is clear; derive the relevant \( z \)-projections of model compartments and perform the integrals in Eq. (6). We return to this more abstract phase of the paper after a practical aside.

5. Flow Diffraction

Artifacts can arise in NMR studies of cells due to their settling (possibly at a uniform rate) in the sensitive volume of the spectrometer probe. Thus the diffusion-diffraction effect could potentially be superimposed upon by a flow-diffraction effect [28-31]. To study this phenomenon and determine its likely influence in experiments on RBCs we built an apparatus that accurately measured the bulk flow on velocity scales that were of the order of only a few cell diameters per second.

In their work on NMR flow-diffraction Callaghan et al. [30,31] first investigated water flow in columns of packed glass and polymer spheres. They mathematically dissected out the contribution of flow from that of the self-diffusion ‘pore-hopping’ [14,15] in the spaces between the spheres that gives interference-like character to \( q \)-space plots; hence they estimated the mean diameter of the spheres by using this theory. They also generated convective flow in a capillary by exploiting the expansion of water that occurred over the length of the sample with a temperature difference of 4°C.

Both of these studies employed an experimental setup that was less direct than what we sought: We used a conventional laboratory peristaltic pump with a bubble trap and a long circuit of 6.5 m of Tygon tubing. This arrangement ensured dampening of any
pulsatile flow introduced by the pump. The tubing was passed down the bore of the NMR magnet, through the probe, and was threaded down the conduit that normally houses the heater coil. De-ionised water of total volume ~60 mL was circulated by the pump with a very stable flow rate. The volume-flow rate of the water was measured using a pre-weighed flask and by recording the volume delivered in 10, 20 and 33 min; thus from measurements of the diameter of the tube the linear flow velocity, \(a\), was calculated.

Figure 3 shows \(q\)-space plots for three different flow rates. The diffraction-like features of the plots are obvious. The linear flow velocity was calculated from the NMR data as follows, and it was based on the assumption that the signal attenuation due to convection (coherent motion) was greatly in excess of that due to self-diffusion (incoherent motion); this is a realistic assumption given that the time over which the motion was measured was \(\Delta = 40\) ms.

\[
\begin{align*}
\text{Fig. 3. Diffraction patterns evident in the } q \text{-space plots of the } ^1H \text{ PFGSE NMR signal from flowing water. Water was pumped up through a high-gradient diffusion probe, in a vertical-bore 9.4 T NMR magnet in the direction of the main magnetic field and field gradients (i.e., } z\text{-gradients). A Gilson Minipuls II pump was used with 2.71 mm i.d. (color code: white/purple) Technicon pump tubing, and the flow-rate settings on the Vernier dial were: A, } '20'; B, '50'; and C, '100'. Independent measurements of linear flow rates were made gravimetrically, using a tared vessel and timing the bulk flow coupled with the measured diameter of the Tygon tubing. The linear flow rates were (\(\mu\)m s\(^{-1}\)): A, 834; B, 1,320; and C, 2,150. The closed water-circuit was via 3.2 mm i.d. clear Tygon tubing, of total length 6.5 m; and a bubble trap was connected immediately after the pump; its total volume was ~2 mL and it contained ~1 mL of air as a 'dampener' of the pulsatile flow that the pump produced [32]. The tubing-circuit was not thermally regulated but the whole assembly was equilibrated within the temperature-controlled spectrometer room. The NMR spectrometer was a Bruker AMX 400 with an Oxford Instruments 9.4 T wide-bore magnet. A Bruker high-gradient diffusion probe (maximum \(\sim 10\) T m\(^{-1}\)) with the gradient along the \(z\)-axis only, and carrying a \(^1H\) exchangeable r.f.-coil insert, was used. The power-supply for the gradient coils was made by Bruker, and the gradient intensity was under software control (\(\text{uxnmr}\)). The pulse sequence was a conventional Carr-Purcell spin-echo with 16-step phase cycle; the field gradients were 4 ms in duration (\(\delta\)) and separated by 40 ms (\(\delta\); and 32 spectra were acquired for each \(q\)-space plot, each derived from summing 64 transients, with an inter-transient delay of 2.8 s.}
6. Analysis of Flow-Diffraction Data

When the distance moved in a given observation time by a liquid flowing in a tube of uniform cross-section is constant across the diameter of the tube, the flow is defined as plug flow; NMR flow diffraction under these conditions, for which dispersion distances due to molecular diffusion are much less than the distances of the bulk flow, has already been thoroughly analyzed [28, 29]. There have been few studies of flow-diffraction since these early reports, and yet it seemed worthwhile to explore some systems again using modern NMR instrumentation, new data processing methods, and in the context of NMR of cells.

The analysis of the data in Fig. 3 can be carried out in a very simple way with little recourse to complex mathematics. It relies on the assumption of plug flow, which as the results show by their consistency with gravitational measurements, pertained in these experiments: Consider a transverse slice of the cylindrical tube inside the sensitive volume of the NMR probe (Fig. 4). The first \( \pi / 2 \) radio frequency pulse in the PGSE experiment nutates the magnetization of the flowing water into the \(-y'\)-axis of the rotating frame. A few milliseconds later a magnetic field gradient pulse of duration \( \delta \) seconds is applied. This advances the magnetization vector in the \( x', y' \)-plane. Thus the gradient pulse can be viewed as a \( z \)-axis pulse whose nutation angle is \( \phi_1 = \gamma \delta g z \), where \( z \) is the axial coordinate in the sample. Next the \( \pi \) pulse rotates the magnetization vector about the \(-y'\)-axis thus bringing about a phase retardation of \(-\phi_1\); and the second field-gradient pulse, applied \( \Delta \) seconds after the first, again invokes a phase change of \( \phi = \gamma \delta g (z + \delta z) \), where \( \delta z \) is the distance moved by the transverse ‘slice’ of water in the time \( \Delta \) between the two gradient pulses. The sum of the phase changes imparted by the two gradient pulses, noting the minus sign introduced by the \( \pi \) RF pulse on \( -\phi_1 \), is simply \( \Delta \phi = \gamma \delta g \delta z \).

Since the uniform linear flow-velocity is \( a \) m s\(^{-1} \) then \( a = \delta z / \Delta \); and by definition \( q = (1/2\pi) \gamma \delta g \) so the change in phase angle brought about by flow is:

\[
\Delta \phi = 2 \pi q \delta z = 2 \pi q a \Delta
\]

The amplitude of the spin-echo signal is described by a squared sinc function of the phase angle \( \Delta \phi \) [28,29]; this is exactly analogous to how the intensity of a light band in an optical diffraction pattern varies as the square of the sinc function of the displacement.
angle of the band relative to the undeviated path of light from a rectangular slit [18]. Hence, the normalized signal $S[q, \Delta]$ is proportional to,

$$S[q, \Delta] \propto \sin^2(2 \pi q a \Delta)/(2 \pi q a \Delta)^2$$  \hspace{1cm} (9)

This function is periodic in $q$ and has minima when the argument is an integer multiple of $\pi$, viz.,

$$q_{\text{min},n} = n/(2a \Delta)$$ \hspace{1cm} (10)

Therefore, experimentally the linear-flow velocity $a$ is estimated from,

$$a = n/(2q_{\text{min},n} \Delta)$$ \hspace{1cm} (11)

In Fig. 3A the first (and only) minimum in the curve is at $q_{\text{min},1} = 1.54 \times 10^4$ m$^{-1}$, and using $\Delta = 0.04$ s this yields via Eq. (11) a flow-velocity estimate of $8.12 \times 10^{-4}$ m s$^{-1}$; this value compared well with the gravimetrically-determined value of $8.34 \times 10^{-4}$ m s$^{-1}$. In Fig. 3B the minima are at $q_{\text{min},1} = 0.94 \times 10^4$ m$^{-1}$ and $q_{\text{min},2} = 1.97 \times 10^4$ m$^{-1}$ which corresponds to $a = 1.33 \times 10^{-3}$ m s$^{-1}$ and $1.27 \times 10^{-3}$ m s$^{-1}$, respectively, which also compared well with the gravimetric estimate of $1.32 \times 10^{-3}$ m s$^{-1}$. Finally, the three flow-diffraction minima in Fig. 3C yielded estimates of $a$ of $2.10 \times 10^{-3}$ m s$^{-1}$, $2.10 \times 10^{-3}$ m s$^{-1}$, and $2.09 \times 10^{-3}$ m s$^{-1}$, respectively, that were very similar to the gravimetric estimate of $2.15 \times 10^{-3}$ m s$^{-1}$.

In conclusion, a simple experimental arrangement was shown to be amenable to the measurement of slow lamina-plug flow rates, using $q$-space analysis. The results give insight into those rates of cell sedimentation that might distort the diffusion-diffraction $q$-space plots obtained from suspensions of RBCs. Our experience with human RBCs suggests that the avoidance of cell settling is easily achieved by using haematocrits greater than 65%; and there does not appear to have been any evidence in our previous work of a settling artifact in the analysis of RBC shapes [11,12,16]. In addition our experimental arrangement is suitable for work with flowing cells.

7. Model of Cellular Arrays

There is interest in defining a system of ordered prisms that might serve as a plausible representation of an array of cells in a suspension or a tissue; the ordered columns of cells around the central sinusoid in the mammalian liver is particularly striking in this regard. By using a model in which dimensions of compartments ($a$) were such that the diffusion distance for water molecules at room- or body-temperature would make $a^2/(\Delta D) \ll 1$ in a PGSE experiment, then the theory in Section 4 can be applied. If the system were well chosen, analytical expressions for the integrals in Eq. (6) might be obtained. Thus $q$-space plots could be predicted based on the basis of the geometry of the array. We defined an array of cells in such a way that a single adjustable parameter determines the packing density (haematocrit) of the cells. The system was built upon a 2-dimensional square tessellation (crystallographic space group P1) with each cell represented by a block of
four squares (side length \(a/2\)); and with each outer corner able to be moved symmetrically, equally, and radially toward the centre of the block. The extent of this movement determines the relative area and hence volume of the ‘extracellular’ space. Since this is a 2-dimensional array, the columns of cells are taken to be effectively unbounded in the direction orthogonal to \(B_0\). While the columns are unbounded they can be conceived of as having transverse partitions that represent membranes, without affecting the present theory.

**Fig. 5.** Construction element of the octagon-star array of prisms that serve as a model of an ordered biological tissue shown in Figs 6D-F. For simulating \(q\)-space diffusion experiments the direction of \(B_0\) was taken to be along the \(z\)-axis. The square that forms the basis of the array of octagons and interstitial stars has side-length \(a/2\); thus the block of four squares has length \(a\); the two lines that define the sides of the four-pointed star intersect at \((a/2, a/2)\) and respectively cut the \(x\)- and \(z\)-axes at \((b, 0)\) and \((0, b)\). Note that \(b < a/2\). In the limit as \(b \to 0\) the octagon-array becomes a square tessellation parallel to the \(x\)- and \(z\)-axes (Fig. 6F); while as \(b \to a/2\) the array is at 45° to the axes (Fig. 6D). For Figs 6A-C the construction element was rotated by 45°.

**Fig. 6.** Canonical, octagon-star arrays used to explore the range of forms of \(q\)-space plots that might be obtained from highly ordered biological tissues, or inanimate materials such as zeolites. A-C, the array orientated with the two diametrically opposite points of each star aligned with the \(x\)- and \(z\)-axes; D-F, the array rotated by 45°. The direction of \(B_0\) was taken to be vertically up the page along the \(z\)-axis.
8. Analytical q-Space Solutions

The simulation of PGSE NMR q-space plots that would be obtained from samples arranged with diffusion restricting barriers (e.g., cell membranes) like those in Fig. 6 is achieved with solutions of Eq. (6). We suppose that the magnetic field gradients occur only along the z-direction so we seek an expression for the probability density \( \rho(z) \) of points from an octagon, and a star projected onto the z-axis. The solution involves the set of integrals that are taken between the straight line segments that define the regions shown in Fig. 5; and we use Euler’s formula to express the exponentials in Eq. (6) as the relevant trigonometric functions. The PGSE signal as a function of \( q \) is Eq. (7) written in terms of the integrals whose subscripts denote the relevant regions:

\[
E[ q, \Delta ]_{\text{total}} = I_{\text{star,}+} + I_{\text{star,-}} + I_{\text{octagon,}+} + I_{\text{octagon,-}}
\]  

(12)

The sub-expression for the star shape in Fig. 6D-F is,

\[
I_{\text{star,}+} = \int_0^b \left( b + z - \frac{2b}{a}z \right) \cos[2 \pi q z] \, dz + i \int_0^b \left( b + z - \frac{2b}{a}z \right) \sin[2 \pi q z] \, dz \\
I_{\text{star,-}} = \int_0^a \left( a - z - \frac{2b}{a}z \right) \cos[2 \pi q z] \, dz + i \int_0^a \left( a - z - \frac{2b}{a}z \right) \sin[2 \pi q z] \, dz
\]

(13)

and for the octagon it is,

\[
I_{\text{octagon,}+} = \int_0^a \left( a - z - \frac{2b}{a}z \right) \cos[2 \pi q z] \, dz + i \int_0^a \left( a - z - \frac{2b}{a}z \right) \sin[2 \pi q z] \, dz \\
I_{\text{octagon,-}} = \int_0^b \left( b + z - \frac{2b}{a}z \right) \cos[2 \pi q z] \, dz + i \int_0^b \left( b + z - \frac{2b}{a}z \right) \sin[2 \pi q z] \, dz
\]

(14)

noting that the haematocrit that we can vary and thus change the form of \( E[ q, \Delta ] \) is given by:

\[
Ht = \left( 1 - \frac{b}{a} \right) \quad b < \frac{a}{2}
\]

(15)

The integrals in Eqs (14) and (15) are not intrinsically difficult to solve but the task is extremely tedious to do ‘by hand’. Thankfully, they are readily solved symbolically (analytically) by using the function Integrate in *Mathematica* [23]. To convey an impression of the complexity of the expressions that arise from the analysis, that for the signal from the stars in Fig. 6D-F is given by:

\[
355
\]

355
\begin{equation}
E_{\text{star}}[q,\infty] = \frac{1}{8a^2} \left( \frac{\pi}{q} \right)^2 \\
\left( a^4 - 4a^2b + 20a^2b^2 - 32ab^3 + 16b^4 + 2a^2b^2 \pi^2 q - a^2b^2 \pi \cos[2b\pi q] + \\
4(a-b)b \left( a^2 \pi - a \cos(a-2b_\pi) + 2(a-2b)\pi \sin(a_\pi) \right) + \right) \\
2a^3(\pi a_\pi) \sin[2b_\pi] \right) 
\end{equation}

(16)

In the interests of conserving space the other expressions are not given here but they can be obtained from the corresponding author. While it is possible to derive satisfaction from writing an analytical solution to the problem, in practice the mathematics only becomes useful when it is coupled with a convenient means of rapid evaluation and graphical output. Accordingly we simulated and graphed the behaviour of restricted diffusion of a molecule like water by varying both \( q \) and \( Ht \) using the 3-dimensional graphics function ListPlot3D in Mathematica (e.g., as in [33]).

Figure 7 shows the dependence of the normalized spin-echo signal intensity \( E[q, \infty] \) from a PGSE NMR experiment, that would be obtained from water diffusing in an array of cells that have an octagonal cross-section with the main diameter similar to that of a typical cell, 10 \( \mu \)m. The range of \( q \) was chosen to be the same as used in many real experiments (see Figs 1 and 2) on RBCs, \textit{viz.}, 0 - 10\(^6\) (m\(^{-1}\)). It is clearly seen in Fig. 7A that as \( Ht \) is increased, making the interstitial space smaller and the star shape more pointed, that the features of the diffraction pattern become better defined. This is in contrast to the situation in Fig. 7C which shows the opposite effect. Another, underlying difference is that the array has been rotated through 45° with respect to \( B_0 \) thus producing the marked change in the features of the diffraction surface.

Figures 7B and D are views from a different perspective of the surfaces; these give a better impression of the form of the \( q \)-space plot when \( Ht = 100\% \). Thus another feature is apparent: the number of lobes in Fig. 7D at \( Ht = 100\% \) is greater than in Fig. 7B. This comes about because the maximum projection of an octagon onto the \( z \)-axis in the former is greater than for the latter; and since \( q \) defines a \textit{reciprocal-distance} space the \( q \)-period is less. This is also apparent, but less clearly, for \( Ht = 0 \) in Figs 7A and C.
Fig. 7. $q$-Space plots calculated for the octagon-star arrays in Fig. 6, for a range of packing densities (haematocrits; $H_t$) of the octagons shown in Fig. 6. For the series in the array in Figs 6D-F where $B_0$ was assumed to be parallel to the axis between pairs of points of each star, A is the view of the three-dimensional surface from the side where $H_t = 0\%$; B, from the side where $H_t = 100\%$. For the series in Fig. 6A-C, C is the view of the three-dimensional surface from the side where $H_t = 0\%$; D, from the side where $H_t = 100\%$. The calculations were made with Mathematica.

Fig. 8. Simulated $q$-space surfaces obtained for a range of packing densities (haematocrits; $H_t$) of the stars alone, as in Fig. 6D-F, assuming no NMR signals emerge from the octagons. When $H_t = 0$ the star is a square with one of the main diagonals along the direction of $B_0$; when $H_t$ is just $< 100\%$ each star is a cross with sharp points, so in a real experiment the signal would be very weak compared with the case when $H_t$ is a smaller value.
Figure 8 shows the form of the $q$-space surface for the situation in which signal is only from the star-shaped compartments; this is the analogue of the intercellular space in our model. Such a plot could come about in real systems when a membrane-impermeant labeled solute is used in the experiment. Note the dimples in the surface between the two sets of intersecting spatial waves. These and their positions are related to the interplay between the projections of the points of the stars and that of its central body. Further insight into the origins of the features could come about by studying the star-shape alone and varying the relative proportions or each feature, unconstrained by the presence of the octagons.

9. Conclusions

We have reviewed the state-of-the-art with respect to the resolution of features in PGSE NMR $q$-space plots that are obtained from suspensions of fresh human RBCs. The signal-to-noise ratio available in a modern NMR spectrometer operating at or above 400 MHz for protons, and equipped with a commercial pulsed-field-gradient probe should reproducibly yield results like those in Fig. 1, in ~30 – 60 min. The application of feature-enhancement strategies like that used for Fig. 2 appear to be a valuable tool for tracking changes of cell shape, such as with the discocyte-echinocyte-spherocyte transition that occurs over a time course of several hours. However, in order to ‘catch’ a time course of the shorter time scale of the first part of this transition, some of the newer fast-DOSY experiments will need to be employed, or specialized versions developed (see our paper on pages 52-68).

The short gradient pulse (SGP) theory of the $q$-space experiment is valuable in conveying an understanding that has close analogies with diffraction and interference effects in physical optics, that have been extensively analyzed [e.g.,18]. The ability to obtain analytical solutions to the master equation in this theory (Eq. 6) by using Mathematica (and presumably similar programs like Maple) means that impressions of the factors that characterize the forms of $q$-space plots can be gleaned by repeated simulations. Overall though, we are pessimistic that truly unique signatures in $q$-space plots will ever be able to be found that will define underlying cell shapes or packing arrangements. In other words, the inverse problem does not appear to have a unique solution, at least given the current signal-to-noise ratios that are available in the standard PGSE NMR experiment. On the other hand, with human RBCs there are very clear differences in the $q$-space plots from discocytes and spherocytes [11,12]; and this has already been of some practical experimental utility. Also, curves like Fig. 1 routinely yield robust estimates of the main diameter of the RBCs. What is unique about this result is that the cell diameter is measured at high packing densities, in contrast to light microscopy in which clear delineation of cells requires packing densities < 1%. Such low packing densities obtained by dilution in buffers are often accompanied by artifacts due changes in osmolality, and contact between the RBCs and the charged surface of the microscope slide that induce shape changes.

The flow-diffraction analysis that we present is applicable only to plug flow. The complete theory has been presented elsewhere; it addresses the paraboloidal flow-front
that is well known for fluids in non-turbulent, Poiseuille flow [28,29]. However, our data reveal the high quality of flow-diffraction data that are available with our simple apparatus in a modern NMR spectrometer. The main conclusion relevant to the studies on cells is that flow-diffraction is not a significant source of error in $q$-space analysis of RBCs in suspensions where $Ht$ is > ~65%, or, of course, if the densities of the medium and the cells are matched.

Finally, PGSE NMR $q$-space analysis provides another window into microscopic systems that contain uniform repeated structures; and it is most informative in cases where the underlying restricting barriers are of the same size (like RBCs) so periodicities in the $q$-space plots become well defined. Then the data can be image-enhanced and a robust representation of the average propagator obtained. Future developments in this area will probably be in more rapid acquisition of $q$-space plots to study systems that are rapidly evolving.

Acknowledgements

The work was supported by a Discovery Grant from the Australian Research Council to PWK. Dr Bob Chapman is thanked for contributions to the experiments for Fig. 3 and for his long-term participation in the NMR work of our group.

References

Modeling Brain Extracellular Space from Diffusion Data

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Abstract

The extracellular space (ECS) of the brain is a thin region surrounding each cell that is filled with a medium resembling cerebrospinal fluid and an unknown amount of extracellular matrix. The ECS is difficult to study but diffusion measurements based on a point-source diffusion paradigm have begun to reveal the complex structure of this region. Despite the complexity, a modified version of Fick’s classical diffusion equation incorporating parameters for volume fraction and tortuosity has been shown to be valid. Using real-time iontophoresis and the small molecule tetramethylammonium, the volume fraction of typical brain tissue has been determined to be 0.2, i.e. 20% of the brain is ECS and the typical tortuosity is 1.6, which means that a small molecule has an effective diffusion coefficient that is 2.6 less than in free solution. Monte Carlo modeling, however, shows that a simple ensemble of convex cells, each surrounded by a uniform ECS cannot generate a tortuosity greater than 1.225. Further modeling suggests that the discrepancy between experiments and theory may be accounted for by the existence of dead-space microdomains in the ECS; a viscous extracellular matrix might also play a role. Diffusion measurements with integrative optical imaging of fluorescent macromolecules and quantum dots show that tortuosity is increased with macromolecular size and analysis based on the theory of restricted diffusion in pores suggests that the width of the ECS is in the range 38-64 nm.

Key words: Brain tissue, extracellular space, volume fraction, tortuosity, dead-space microdomain, extracellular matrix, tetramethylammonium, dextran, quantum dot

1. Introduction

The brain enables us to analyze complex scenes and sounds in a fraction of second and is responsible for the performance of exquisite movements orchestrated by a vast array of coordinated muscles. Beyond that, the brain is the source of speech, reasoning, memory and consciousness, but ideas about how this organ works remain very rudimentary. Yet the brain is no more and no less than a vast assemblage of communicating cells. These cells have a complex geometry and are closely packed but they maintain a small space between them, the extracellular space (ECS). This space is vital to brain function and it is equally important as a conduit for the delivery of therapeutic drugs. Unfortunately the ECS is hard to study. By analyzing the diffusion of molecules in the ECS [1, 2] it is possible to build up a picture of the ECS and models are emerging. These models describe different aspects of the experimental data and the challenge for the future is to arrive at a comprehensive description.
2. Extracellular space

The ECS has been likened to the “water phase of a foam” [3] with the foam representing the cells. This is a useful but simplistic image. The cells of the brain are actually divided into two types, neurons that communicate by means of electrical and chemical signals and glial cells that are equally numerous but whose overall function remains unclear. Both these types of cell have rounded bodies containing a nucleus but they also have long and often branched extensions that are cylindrical in cross-section for neurons but may be sheet-like for glia. The net effect is a complex ‘neuropil’ (Fig. 1) containing profiles that range in size from less than a micrometer to tens of micrometers. Despite the complexity, the ECS has many of the characteristics of a porous medium and relevant theories from other disciplines can be employed.

In Fig. 1 the ECS has been filled in (black lines) and it is evident that every cell membrane is surrounded by a thin ‘atmosphere’ of ECS. This atmosphere contains a fluid that is similar in composition to the cerebrospinal fluid that bathes the outer surface of the brain and is present in large internal cavities or ventricles and in the spinal canal. This fluid is predominantly composed of sodium and chloride dissolved in water with much smaller amounts of other substances, including potassium and calcium. There is another entity in the ECS and that is the extracellular matrix. This matrix is composed of long-chain molecules that have many negative charges. Among the main components are hyaluronan and chondroitin sulfate [4]. One problem is that it is not known how much matrix is present in the ECS and it probably varies with different brain region. If there is a lot of matrix then it will increase the viscosity experienced by a diffusing molecule.

3. Diffusion concepts applied to the ECS

Because the ECS is essentially a fluid-filled space it does not survive well the tissue processing that is required to produce an electron micrograph and typically the width of the space is reduced. But beyond that, the dynamic properties of the ECS – how it influences molecular transport for example – cannot easily be estimated from 2D fixed sections of the brain. This type of information can be revealed from diffusion measurements.
Early work on diffusion in the brain used radiotracers, such as sucrose, that could be expected to remain largely confined to the ECS. Typically, one surface of the brain was bathed in the tracer for some time, allowing the substance to penetrate and form a concentration gradient. Then the tissue was fixed and cut as a sequence of small blocks perpendicular to the surface, the tracer in each was quantified and the diffusion properties of the brain region estimated [1, 5].

More recent diffusion measurements have used variations of a ‘point-source paradigm’, largely developed in our laboratory, to reveal the ‘real-time’ diffusion properties of small regions of brain tissue (Fig. 2). The crux of this method is to use a glass micropipette with a tip diameter of only a few micrometers to release a small quantity of a substance into the ECS and then monitor the subsequent concentration of the substance as a function of time and position. This paper will be based primarily on data obtained with such a paradigm, but the results are in agreement with the radiotracer studies.

The fundamental hypothesis behind all these measurements is that small substances that enter the ECS and remain confined to this domain will move predominantly by diffusion. Because diffusion is a macroscopic expression of an ensemble of microscopic random walks, molecules will explore the structure of the ECS and the concentration distribution will reveal important parameters of the local structure [2]. The most important parameters are volume fraction and tortuosity.

For an ideal molecule, i.e. one that stays in the ECS, volume fraction (\(\alpha\)) is defined as

\[
\alpha = \frac{V_{\text{ECS}}}{V_{\text{Tissue}}}
\]

where \(V_{\text{ECS}}\) is the volume of the ECS and \(V_{\text{Tissue}}\) is the volume of the entire tissue. Both volumes are defined with respect to some Representative Elementary Volume (REV), typically for the point-source paradigm of the order of \(10^6 \, \mu m^3\). If electron micrographs were perfect, \(\alpha\) could be obtained from stereological measurements on images, such as that shown in Fig. 1, by simply comparing surface areas or line segments.

Tortuosity (\(\lambda\)) is a more complex parameter than volume fraction but it is easy to define operationally as
\[ \lambda = \sqrt{D/D^*} \]  

where \( D \) is the diffusion coefficient for the ideal molecule measured in a free solution, often a very dilute agarose gel (0.3\% w/v) made up with a salt solution that mimics the major components of the fluid in the ECS, and \( D^* \) is the effective diffusion coefficient measured in the brain (note that in chemical engineering the tortuosity is generally equated to \( D/D^* \) rather than \( \sqrt{D/D^*} \)).

The governing diffusion equation linking \( \alpha \) and \( \lambda \) is then defined as a modification of Fick’s second law

\[ \frac{\partial C}{\partial t} = \frac{D}{\lambda^2} \nabla^2 C + \frac{Q}{\alpha} - F_{\text{Loss}}(C) \]  

where \( Q \) is a source term and \( F_{\text{Loss}}(C) \) is a term that accounts for any loss of molecules from the ECS. For ideal molecules, loss will be zero. There are several important caveats regarding this equation. The first is that the concentration measured along any line in the tissue is actually discontinuous because the intracellular concentration is zero for an ideal molecule so that the rigorous derivation of the diffusion equation involves a volume-averaging process over the appropriate REV \([1, 6]\). This process also provides a justification for the tortuosity. The second caveat is that in Eq. (3), and throughout this paper, the concentration \( C \) is defined as the actual concentration measured in the ECS. This is the physiologically relevant variable because it is what a receptor on a cell membrane experiences and that is also the concentration measured in most experiments described here. However, other disciplines and some experiments use concentration per unit volume of tissue, \( C_1 \). It is clear that \( C_1 = \alpha C \) but failure to recognize which definition is in use can result in a misinterpretation of the literature. The third caveat is that bulk flow within the ECS has been neglected because it is negligible in the experiments that form the basis of this paper. Bulk flow refers to hydrodynamic flow within the ECS, which is probably confined to a narrow perivascular space around some blood vessels in normal tissue (see \([1]\) for more detail).

Because \( D^* < D \) it follows that \( \lambda > 1 \) and consequently tortuosity may be thought of as a measure of the hindrance that the brain places on a diffusing molecule with respect to a free medium. As will become apparent, however, the interpretation of \( \lambda \) in terms of specific structure is an entirely separate process from its definition given by Eq. (2), so tortuosity is not as conceptually simple as volume fraction.
4. Measurements with small ‘ideal’ molecules

The first realization of the point-source paradigm considered here is the real-time iontophoresis method using tetramethylammonium, abbreviated as the RTI-TMA method. TMA$^+$ is a small cation of 74 molecular weight that approximates an ideal point molecule that may be expected to explore the entire ECS. The concentration of TMA$^+$ as a function of time $t$ at a distance $r$ from the point source micropipette can be sensed with an appropriate ion-selective microelectrode (ISM). A typical experimental arrangement is shown in Fig. 3. The appropriate solution to Eq. (3), described in [1, 6], is

$$C(t) = \frac{Q}{8\pi D^* \alpha r} \left[ \text{erf} \left( \frac{r}{2\sqrt{D^* t}} \right) \exp \left( \frac{k'}{D^*} \right) - \text{erfc} \left( \frac{r}{2\sqrt{D^* t}} \right) \exp \left( -\frac{k'}{D^*} \right) \right].$$

(4)

Some TMA$^+$ is lost from the ECS, either into cells or across the blood-brain-barrier and this is accounted for by setting $F_{\text{Loss}}(C) = k'C$ in Eq. (3). Eq. (4) assumes that the source $Q$, which is a point-source in space, is begun at time $t = 0$ and continues to infinity. To obtain the solution for a finite pulse of duration $t_p$, a delayed form of Eq. (4) must be subtracted from the infinite duration solution

$$C = C(t) - C(t - t_p), \quad t > t_p.$$

(5)

Finally, $Q$ itself is defined in terms of the applied iontophoretic current $I$ and transport number of the source electrode $n_t$ as $Q = In_t F$ where $z$ is the valency of the ion (+1 for TMA$^+$) and $F$ is Faraday’s Electrochemical Equivalent. For a detailed description of the method see [6 – 8].

Non-linear curve fitting of Eqs. (4) and (5) to experimental data obtained in a dilute agarose gel (where $\lambda = 1$, $\alpha = 1$, $k' = 0$) determine $D$ and $n_t$ and then similar curve fitting in brain tissue provides $D^*$ and $\alpha$. From $D$ and $D^*$ the tortuosity is calculated using Eq. (2). The value of $k'$ is also obtained in brain tissue but this is really a correction factor for the non-ideal behavior of TMA$^+$ and not a fundamental parameter so it will not be
considered further here. The data acquisition and analysis are performed by custom software (Wanda and Walter, available from C. Nicholson).

5. Data and models for small ‘ideal’ molecules

From more than 30 peer-reviewed studies with the RTI-TMA method carried out predominantly in our laboratory in New York and the laboratory of Prof. Eva Syková in Prague it can be said that the diffusion equation (Eq. 3) is applicable to brain tissue. Further, it is established that in normal brain tissue $\alpha = 0.2$, i.e. 20% of the brain is actually ECS, and $\lambda = 1.6$, i.e. a small molecule has an effective diffusion coefficient that is reduced by about 2.6 compared to that in a free medium. Of course there are deviations from these values in some regions of the brain, for example diffusion is anisotropic in the cerebellar molecular layer [9] and in fiber tracts of the brain such as the corpus callosum [10] but on the whole the similarities in results are more striking than the differences and the same values occur in a range of species.

5.1 Equally-spaced convex cell models

Can a model account for $\alpha = 0.2$ and $\lambda = 1.6$? The most elementary model is to return to the idea of the ECS as the water phase of a foam but to simplify the problem even further by representing the cells by cubes of uniform size and equal spacing (Fig. 4A). By choosing an appropriate cube size and spacing, $\alpha$ will be specified and then $\lambda$ can be estimated by running a Monte Carlo simulation. For the simulation, a large number of point particles are released from a point source in the middle of an ensemble of many cubes and the particle distribution after a certain number of time steps is measured. It is assumed that each particle moves within the ECS with a diffusion coefficient $D$ while making occasional specular collisions with the walls of the cubes. Because cubes are space-filling, the value of $\alpha$ can be varied from 0 to a value approaching 1 (free medium without cells) and a range of $\lambda$ is obtained. This plan was implemented by Tao & Nicholson [11] using the program MCell developed for simulating some other neurobiological problems [12]. The simulations suggested that a simple relation

$$\lambda = \sqrt{\frac{3 - \alpha}{2}}$$

(6)
exists between $\alpha$ and $\lambda$. Remarkably, this is the same result that was obtained by James Clerk Maxwell in 1891 (See [13]) for a dilute suspension of spheres. Some work has indicated that Maxwell’s result should hold for more densely packed structures [14]. The maximum value of $\lambda$ occurs in Eq. (6) when $\alpha \to 0$ (this is possible because a point particle of negligible size is being used) and in this limit $\lambda \to \sqrt{3/2} = 1.225$. This limiting value was also derived by other investigators using different approaches (e.g. [15 – 17]).

Cubes pack with long aligned channels (Fig. 4A) however, so to confirm that Eq. (6) and its limiting value were not related to this feature, Tao and Nicholson [11] used two other space-filling sets of objects, namely truncated octahedra (Fig. 4B) and a combination of rhombicuboctahedra, cubes and tetrahedra. The results were exactly the same as for cubes and it was hypothesized that the result held for any set of space-filling convex cells that had uniform spacing. A more sophisticated Monte Carlo simulation with pseudo-random shapes further confirmed this [17].

![Fig. 5. Dead-space microdomains. A. 2D representation of a cubic cell with a cavity or ‘pocket’ dead-space. B. Cubic cell partially wrapped by a sheet-like glial cell. The wrapping forms a dead-space. C. Four cubic cells with uniform spacing (no dead-space). D. Four cubic cells deformed to allow a void dead-space to form at center.](image)

5.2 Dead-space microdomain models

The equally-spaced convex cell results raise an important problem when compared with the experimental data showing that usually $\lambda = 1.6$ with $\alpha = 0.2$ because the models described by Eq. (6) give $\lambda = 1.183$ for $\alpha = 0.2$ and the maximum value cannot exceed 1.225. This prompted an examination of the underlying assumptions of the model. The first assumption was that the cells had to be convex. As noted in Section 2, the cells of the brain have a very complex geometry; in 2D sections the majority of profiles can appear convex but may actually be different in 3D. This leads to the hypothesis that concave elements might exist, either as invaginations of a cell membrane (Fig. 5A) or as a more extensive enveloping sheet-like configuration (Fig. 5B). Both these cases amount to dead-space microdomains that are not well-connected with the rest of the ECS. By this it is meant that a diffusing particle upon entering such a microdomain explores it for a while and then exits at the same location that the particle entered. Thus the particle does not advance towards its goal but merely loses time. For suitable geometries and diffusion processes this is known to increase the effective diffusion coefficient (e.g. [18]).
Hrabětová and co-workers [19] have provided experimental evidence for the existence of dead-space microdomains in ischemic brain tissue and it is plausible, but not completely established, that such dead-spaces might exist in normal tissue. A model based on dwell-times was proposed [19] and more rigorously analyzed by Hrabe et al. [17] that yields, for small microdomains and volume fractions in the range encountered in brain tissue, the basic formula

\[ \lambda = \lambda_o \sqrt{\frac{\alpha}{\alpha - \alpha_d}} \]  

(7)

where \( \lambda_o = 1.225 \) is the limiting volume fraction derived from Eq. (6), \( \alpha_d \) is the volume of the dead-space microdomains and \( \alpha \) is the total volume fraction of the ECS. The well-connected extracellular space with volume fraction \( \alpha_o \), is that remaining after elimination of the dead-spaces so \( \alpha = \alpha_o + \alpha_d \). Application of Eq. (7) to experimental results has revealed that the ratio \( \alpha_d:\alpha_o \) would be expected to be about 40:60 in normal cortical tissue and 60:40 in pathological ischemic tissue ([19, 20]).

These results were generalized to larger microdomains and volume fractions [21], based on detailed Monte Carlo simulations, resulting in the expression

\[ \lambda = \left( \frac{\alpha - \alpha_d}{\alpha_o} \right)^{\frac{1}{\beta}} \]  

(8)

where \( \beta \) is an empirical parameter in the range 2 – 3. These simulations also covered the case where the dead-space took the form of a local enlargement of the ECS or a local void (Fig. 5C, D). These voids also delay particles because, once they enter such a region they take some time to find their way out again. This result is known in 2D [22, 23]. The void result deals with the second assumption that was made in the simple model, namely that the spacing between cells remained constant. Introducing local enlargements violates this condition and increases \( \lambda \).

The two cavity models (Eq. 7 and Eq. 8) are plotted in Fig. 6 for fixed values of \( \alpha_d \) along with the cavity-free Eq. (6). For Eq. (8), \( \beta = 2 \) corresponds to a ‘pocket’ (Fig. 5A) while \( \beta = 3 \) is appropriate for a void (Fig. 5C,D); see [21] for more detail. It is evident that the presence of cavities dramatically increases \( \lambda \) as \( \alpha \) diminishes.

![Fig. 6. Tortuosity as a function of \( \alpha \) for a fixed \( \alpha_d \). For Eq. (7) \( \alpha_d = 0.083 \); for Eq. (8), \( \beta = 2, \alpha_d = 0.091 \), and \( \beta = 3, \alpha_d = 0.119 \). These values ensure that when \( \alpha = 0.2, \lambda = 1.6 \). Eq. (6), the case with no cavities, is plotted for comparison.](image-url)
It is worth noting that the limiting value of $\lambda$ for 2D ensembles of squares or other convex structures is $\sqrt{2} = 1.414$ [15], which is greater than the value of 1.225 that applies to 3D convex cells. Furthermore, if the ECS is composed of connected tubes instead of the planes that underlie Eq. (6), the limiting value of $\lambda$ is $\sqrt{3} = 1.732$ [15]. Thus the tortuosity is a subtle measure of connectivity and geometry and this makes it difficult to estimate it from simple models because they often fail to take into account all the possible pathways, or local delays, inherent in the geometry [17, 23]. Indeed, Torquato [24] has argued that the sort of random media under discussion here require an infinite set of $n$-point correlation functions in order to characterize them completely.

5.3 Extracellular matrix models

The previous arguments show that the basic geometrical tortuosity associated with an ensemble of uniformly spaced convex cells can be increased by introducing a more complex geometry and this strategy will be capable of elevating the value of $\lambda$ to 1.6 or more for a volume fraction of 20%. This is not, however, the only way in which tortuosity might be increased; the extracellular matrix could also accomplish this. If the matrix is regarded as a polymer solution then a large literature may be applied to its representation [25, 26]. An attempt to model both the geometrical and viscous components of tortuosity in brain tissue was made by Rusakov & Kullmann [27]. But because so little is known about the extent of the matrix, such elaboration may be unwarranted and simpler models suffice that just represent the viscosity of the ECS as greater than that of a free solution. Because $\lambda$ can always be formally decomposed into a set of multiplicative components

$$\lambda = \frac{D}{D'} = \sqrt{\frac{D_1 D_2 \ldots D_{n-1} D_n}{D_{n-1} D_n}}$$

it is plausible that $\lambda$ can be expressed as the product of a geometrical tortuosity described above and a viscous component i.e. $\lambda = \lambda_{geom} \lambda_{visc}$. It has also been suggested from physiological experiments that the introduction of 5% 40 kDa dextran into the ECS increases ECS viscosity [28] so an intrinsic matrix might have a similar effect.

6. Measurements with macromolecules

While TMA$^+$ and some other small ions have been excellent probes of the ECS they do not tell us how a much larger molecule would behave. Many important signaling agents in the brain are macromolecules (e.g. nerve growth factors). Furthermore, the diffusion of large molecules can reveal new properties of the ECS; the latter perspective is emphasized here.

The RTI method used with TMA$^+$ cannot be applied to macromolecules both because it is usually difficult to iontophorese large molecules and because it is not possible to fabricate ISMs that can sense macromolecules. To study macromolecules, Nicholson and Tao [29] introduced a variant of the point-source paradigm which they called Integrative Optical Imaging (IOI).
In the IOI method (Fig. 3), macromolecules carrying a fluorescent label are released from a micropipette by a short pressure pulse and the resulting diffusing cloud of molecules imaged using a conventional epifluorescent microscope equipped with a digital camera connected to a PC [29, 30]. If the pulse is very brief compared to the duration of subsequent diffusion processes, then it can be regarded as a delta function in both time and space and the appropriate solution (see [1, 29]) to Eq. (3) is:

\[
C(r, t_i) = \frac{UC_f}{\alpha} \frac{1}{\left(4D^*(t_i + t_0)\pi\right)^\frac{3}{2}} \exp\left(-\frac{r^2}{4D^*(t_i + t_0)}\right),
\]

where a volume \( U_c \) of the macromolecular solution at concentration \( C_f \) is ejected. The variable \( t_0 \) represents a virtual source time origin such that the source appears to have been activated at time \( t_0 \) before it actually occurred. This allows a point-source formalism to be employed even when a finite initial volume is released (see [31]). Because large molecules do not easily leave the ECS, no loss term is needed, i.e. \( F_{\text{Loss}} = 0 \) in Eq. (3).

To make use of Eq. (10) it is necessary to relate the image intensity distribution recorded by the camera to the concentration. The theory of how the image of the diffusing cloud of molecules maps onto the plane of the camera is complicated [29, 32] but it may be shown that Eq. (10) can be reduced to Eq. (11). In both Eq. (10) and Eq. (11) a discrete time \( t_i \) is used to represent the sequence of camera images:

\[
I_i(r, \gamma_i) = E_i(\gamma_i) e^{-\gamma_i r_i^2}, \quad \text{and} \quad \gamma_i^2 = 4D^*(t_i + t_0)
\]

where \( I_i \) is the intensity of the fluorescence and \( E_i \) is an amplitude term embodying the defocussed point-spread function of the objective [29, 32]. By fitting the exponential term in Eq. (11) to the spatial distribution at a sequence of times \( t_i, D^* \) can be determined [29]. Control diffusion measurements to determine \( D \) are made in dilute agarose and then \( \lambda \) is calculated from Eq. (2). The actual data analysis is carried out using custom software (Vida and Ida, available from C. Nicholson and described in more detail in [30]).

7. Data and models for macromolecules

The diffusion of a variety of globular and flexible chain molecules in brain tissue has been reported in several papers. Here attention will be confined to molecules with a globular structure where a meaningful hydrodynamic diameter can be estimated from the free diffusion coefficient using the Stokes-Einstein equation [33]

\[
d_H = \frac{k_B T}{3\pi \eta D} \times 10^{13}
\]

where \( d_H \) is the molecule diameter (nm), \( k_B \) is Boltzmann’s constant \((1.38065 \times 10^{-23} \text{ J.K}^{-1})\), \( T \) is temperature (K), \( \eta \) is viscosity (Pa.s) in the solvent (usually water) and \( D \) is the free diffusion coefficient (cm$^2$ s$^{-1}$).
Two types of globular macromolecules may be considered: dextrans and proteins. Dextrans are aggregates of long-chain sugar molecules that form a loose ball in solution and only have an approximate molecular weight. In contrast, proteins are rigid molecules with a well-defined structure, parts of which may make specific interactions with receptors on cells or components of the extracellular matrix. For the protein studies referenced here, such specific interactions are either non-existent or have been suppressed, so both types of molecule probe the more general properties of the ECS.

Using dextrans ranging from 3 kDa to 70 kDa and proteins ranging from 6.6 kDa to 66 kDa in slices of rat brain it is a general finding that the measured tortuosity is no longer 1.6 but ranges from about 1.7 – 2.5 and there is a steady increase in $\lambda$ with molecular weight (Fig. 7). Thus larger molecules are more hindered, probably through increasing interaction with the membranes that define the ECS.

7.1 Models to estimate the width of the ECS

The increase in tortuosity with molecular size prompts the question of what is the largest molecule that can diffuse through the ECS. In principle one could answer this by selecting a range of molecules of ever-increasing size until one was reached that no longer diffused. In practice, however, this is not possible because the time needed to determine the effective diffusion coefficient in the tissue would eventually exceed the viable lifetime of the biological preparation, as the chosen molecules diffused ever more slowly. To make practical measurements, Thorne and Nicholson [38] turned to the theory of restricted diffusion (RD) in narrow pores and probed the diffusion properties of the anesthetized in vivo rat cortex with a recently developed nano-particle, the quantum dot.

Quantum dots (QDs) are nanocrystals that have a core which emits fluorescent light at a precise frequency. The core is enclosed in a protective shell and, for use in the diffusion studies, the shell must be coated with short strands of polyethylene glycol (PEG) to make the QD water soluble and inert. A QD with a final diameter of 35 nm (QD655, manufactured by Invitrogen, Carlsbad, California, USA) was chosen and diffusion measurements were made with the IOI technique along with measurements of 3 and 70 kDa dextrans. The QD did diffuse but with $\lambda = 10.6$, a value of unprecedented magnitude.
To interpret these results in terms of the width of a channel required to allow a QD to diffuse, a similar decomposition of the tortuosity to that described for the matrix (Eq. 9) may be used

\[ \lambda = \sqrt[3]{\frac{D}{D'}} = \sqrt[3]{\frac{D'}{D}} = \lambda_{\theta=0} \sqrt{\frac{D}{D'}} \]  

(13)

where \( D' \) is the interstitial diffusion coefficient. For neutral, inert substances subject to purely steric interactions with pore walls, \( D'/D \) only depends on channel geometry and \( \theta \), the ratio of probe hydrodynamic diameter, \( d_h \) (Eq. 12) to brain ECS width, \( d_{ECS} \), i.e. \( \theta = d_h/d_{ECS} \). The parameter \( \lambda_{\theta=0} \) is the tortuosity for a vanishingly small molecule.

Broadly speaking there are two types of RD model that might apply to the ECS, a plane model or a cylinder model (Fig. 8). The previous discussion makes it plausible that the ECS is a set of intersecting planes and therefore the theory developed by Deen, [39] would be appropriate. However the actual space in which a large molecule moves might be reduced to a set of interconnected tubes or cylinders because of the presence of the matrix. To a macromolecule the ECS might appear as a set of connected tunnels through the matrix, then the theory of Bungay & Brenner, [40] would be appropriate. In either case, appropriate expressions for \( D'/D \) can be inserted into equation (13) resulting in two free parameters, \( d_{ECS} \) and \( \lambda_{\theta=0} \). Taking the example of parallel plane geometry [39]:

\[ \sqrt{\frac{D}{D'}} = \left[ (1-\theta)(1-1.004\theta + 0.418\theta^3 + 0.210\theta^4 - 0.169\theta^5) \right]^{1/2}. \]  

(14)

Non-linear least squares fitting of Eq. (13), substituted with Eq. (14) to the results (Fig. 9), as well as fitting the more complicated expression for a cylindrical pore [40] led to estimates for \( d_{ECS} \) and \( \lambda_{\theta=0} \) [38]. RD theory fit the tissue data well for both models yielding \( d_{ECS} = 37.7 \) nm and \( \lambda_{\theta=0} = 1.72 \) for parallel planes and \( d_{ECS} = 63.8 \) nm and \( \lambda_{\theta=0} = 1.63 \) for cylindrical pore geometry [38]. At this time there is insufficient data to choose between the two models so it can only be said that these results suggest that the width of the ECS is between 38 and 64 nm.

These results challenge the long-held view that the width of the ECS is about 20 nm. The 20 nm estimate was based on electron micrographs, however it is well established that conventional electron microscopy tends to obliterate the ECS (see [38] for a summary of the literature) so the new estimate is not implausible. This finding has importance not only for understanding how large an effective signaling molecule can be in the ECS, but also for the design of drug delivery strategies that may employ antibodies, viral vectors or other large vehicles.
7. Other modeling issues: binding, uptake and charge

This brief review has focused on physical impediments to diffusion in the ECS but there are other mechanisms that may determine the ability of a molecule to reach a given destination. Chief among these are the possibilities that a specific molecule may bind to a receptor on a cell membrane or be taken up into a cell. Indeed, all molecular signals must eventually undergo this fate in order to be effective.

This mechanism was represented by the general term $F_{\text{Loss}}$ in Eq. (3). When the binding or uptake is irreversible and proportional to the concentration, an analytical solution to Eq. (3) often may be found. This is exemplified by the solution for the RTI-TMA method described by Eq. (4). This type of kinetics is also appropriate for molecules that are lost into the blood stream across the blood-brain barrier [41]. In many instances of biological interest, however, the binding or uptake process saturates leading to Michaelis-Menten kinetics and non-linear solutions to the diffusion equation. An example of this is the behavior of the neuromodulator dopamine in certain brain regions [42]. Thus in some situations the transport process may be dominated by binding, uptake or loss rather than diffusion.

Finally, if the binding is reversible and the process is brief compared to the general diffusion process, the effect is simply an increased tortuosity [43]; this is essentially the case with small dead-space microdomains and the reason why it is legitimate to employ the diffusion equation for such problems. Some form of fast reversible binding also may underlie non-specific interactions of charged molecules with the fixed negative charge groups on the extracellular matrix; but this interaction is poorly understood at present.

8. Conclusion

Diffusion measurements in living brain tissue have revealed that the ECS is a more complex microenvironment than hitherto thought. The geometry may harbor dead-space microdomains, a matrix within the ECS may impede molecular movement and the transport of large molecules may be dominated by the drag of the cellular membranes that form the boundary of the ECS. For some molecules, other effects such as binding, loss or charge interaction may play a major role. So the ECS has the potential to channel molecular signals or be permissive to some but restrictive to others and this behavior may vary from brain region to region. In order to understand these effects and their...
implications, models are essential and a few have been described here. Other models have been published (e.g. [17, 27, 44, 45]) and modeling the ECS seems a fertile area for physicists and engineers in the future. All modeling, however, needs to be done in concert with careful experiments because Nature never ceases to surprise us!

Acknowledgement

This review is largely based on the careful experimental and theoretical work of my colleagues, Jan Hrabe, Sabina Hrabětová, Lian Tao and Robert G. Thorne. I thank Jyoti Patel for comments on the manuscript. The work was supported by Grant NS-28642 from the US National Institute of Health.

References


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Invasions of Isotopes and of Neobiota

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Extended abstract

Abstract

We report on invasions with low diffusivity: one in materials science and one in ecology. What is interesting in materials science is to describe diffusivities in order to model technological important materials. In ecology on the other hand predictions into the future appear the most challenging issue.

Keywords:
Invasions, isotopes, neobiota, diffusion in materials science, diffusion in ecology, Mössbauer effect at synchrotron, warming of climate, Monte Carlo method

1. Materials science:

X-radiation from synchrotrons and in the future from free electron lasers offers a number of new possibilities for diffusion studies [1]. Here we report on a new method which takes advantage of the high brilliance of synchrotron radiation over a broad energy range. Due to its brilliance synchrotron radiation can excite even the energetically very narrow energy levels (here the first excited level of $^{57}$Fe) as has before only been possible by the Mössbauer effect. The radiation is reemitted with delay (the life time of the nuclear level) and can be used for diffraction studies. In the work reported here our aim was to understand diffusion in FePt which is a main candidate for next generation data storage devices. We have studied diffraction from the superstructure Bragg-peak of a multilayer sample composed of layers of FePt with the iron isotope alternating between natural iron and $^{57}$Fe. Diffusion of iron (the “invasion” of $^{57}$Fe isotopes into layers with natural iron and vice versa) leads to a disappearance of the preponderance of the $^{57}$Fe isotope concentration in every second layer and therefore to a decrease of the height of the Bragg peak. In this way Fe diffusivities in the range of 10⁻²⁴ m²/s can be determined which is less than with any other method [2].
2. Ecology:

Ambrosia artemisiifolia, the American ragweed, is a short-lived vascular plant species which has been invading Central Europe for 150 years, but caused by the warming of the European climate [3] its spread process has accelerated in the last few decades. The pollen of ragweed evokes heavy allergies and – what probably counts even more – because of its spread rather late in summer causes a second wave of allergy when other pollen allergies have decayed. Since both historical distribution and spread of Ambrosia artemisiifolia are well documented, Ambrosia artemisiifolia is perfectly qualified as model species. We have tried to reconstruct the invasion process of Ambrosia artemisiifolia in Austria by collecting all records. By correlating distribution data and data sets on land use, road and railway networks, and climatic variables, a habitat layer for Austria was produced which is implemented in the simulations. Comparison of the observed and the modelled historical diffusive spread of Ambrosia artemisiifolia from 1990 to 2005 is used to test accuracy and applicability of the Monte Carlo method [4]. Furthermore, including regional climatic change scenarios yielded new habitat layers for increased July mean temperatures of up to +4.5°C. These new habitat layers can also be used for the simulations, enabling dispersal predictions with respect to different climatic scenarios.

References
Diffusion in Silicate Melts: Kinetics and Mechanisms of Redox Reactions

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Diffusion in silicate melts plays a fundamental role in all magmatic processes in nature as well as in the glass industry. Of particular importance is the diffusivity contrast that occurs between the so-called network-former (e.g., Si, Al) and network-modifier (e.g., alkali and alkaline-earth) cations. Whereas the diffusivities of all these cations tend to converge at the high-temperature limit, a strong decoupling is observed when the glass transition is observed. Scaling with the viscosity of the melt, the diffusivity of oxygen and network-former cations then becomes much lower than that of network-modifier cations.

This decoupling has considerable influence on crystallization because the crystals that precipitate irreversibly at strong degrees of supercooling are nonstoichiometric and show anomalously high concentrations of the most mobile elements. This decoupling also exerts a very strong influence on the kinetics and mechanisms of redox reactions. In both instances the mechanisms that operate do not ensure minimization of the Gibbs free energy of the system, but allow it to decrease fastest to a point where the system becomes trapped in a metastable state.

In redox reactions, this feature means that diffusion of oxygen is the rate-limiting factor only at superliquidus temperatures. At lower temperatures, the kinetics of these reactions is controlled instead by diffusion of alkaline-earth or alkaline cations coupled to a flux of electron holes. For iron redox reactions, we have investigated these effects quantitatively from the glass transition up to 2100 K by in situ X-ray absorption Near Edge Structure (XANES) experiments at the iron K-edge. Near the glass transition, similar kinetic experiments have yielded the same results. To rationalize in a simple way the observations made, we have introduced the concept of redox diffusivity from the time required to achieve redox equilibrium at a given temperature. Comparisons of these redox diffusivities with the diffusivities of oxygen, network-forming and network-modifying cations then allow one to distinguish the temperature range where a given redox mechanism predominates. The results obtained in this way for a variety of alkali and alkaline-earth iron silicates will be presented.
Intermittent Brownian Dynamics over Strands

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1. Introduction

Intermittent dynamics is intrinsically involved for particles or molecules exploring confining interfacial systems such as porous material, colloidal suspension and catalytic surfaces. Periods of relocation in the bulk phase or in the pore network (bridges) alternate with adsorption or trapping steps generally located nearby the interface (cf. Fig 1A). A representative example concerns the search for a specific target site on DNA by a protein, which alternates adsorption or scanning phases where the protein diffuses on the DNA strand and three-dimensional bulk excursions or relocations [1]. A better understanding of such dynamics is needed in order, for example, to optimize the intermittent search strategy [2]. Recently [3], we have proposed a theoretical analysis of field cycling NMR dispersion technique (NMRD) experiments allowing to probe the time/frequency dependence of relocation steps [4] and adsorption periods of an intermittent fluid dynamics near an interface. In this communication, we consider the Brownian dynamics of a fluid molecule over thin and very long mineral strands having a diameter of about 3 nm. Experiments are compared with numerical simulations and theoretical derivation.

2. Probing relocation statistics over strands by NMR relaxometry


In the following, we consider the case of imogolite particles in very diluted suspensions. Imogolite are thin and very long cylinders having a diameter similar to DNA
molecules. Using NMFD, we observe characteristic dispersion curves, $R_1(\omega)$, vastly
different from that found for flat or curved interface[5]. As shown in Fig 1B, $R_1(\omega)$
evolves $-\ln(\omega)$ at low frequency. A crossover to a $1/\omega$ is observed above 1 MHz.
Brownian dynamics simulation is found to be in good agreement with experimental
results, as show in Fig 1B.

3. Theoretical analysis.
We have shown [3] that an intermittent Brownian dynamics induces a low
frequency dependence of $R_1(\omega) \propto J(\omega)+4J(2\omega)$ with:

$$J(\omega) = \frac{1}{\omega} \text{Re}\left(\frac{(1-\tilde{\psi}_h(\omega))(1-\tilde{\psi}_a(\omega))}{1-\tilde{\psi}_h(\omega)\tilde{\psi}_a(\omega)}\right)$$  \hspace{1cm} \text{Eq}(1)

$\tilde{\psi}_h(\omega)$ and $\tilde{\psi}_a(\omega)$ are the Fourier transforms of the probability density function
characterizing the relocation and the adsorption step, respectively. Using classical
properties of the potential theory in 2D, it is possible to show that for a cylinder, at long
time, we have $\psi_h(t) \propto 1/t(\ln^2(t))$. The $1/t$ evolution determines a $-\ln(\omega)$ evolution of
$J(\omega)$ at small frequency. At short time, the diffusor can only probe the local “flat” surface
of the cylinder and then evolves as $1/t^{3/2}$ [4]. This last property induces an algebraic decay
of $J(\omega)$ at large frequency [3,5]. These two theoretical results explain in a closed form the
experimental data.

4. Conclusion
We have investigated the slow fluid molecule dynamics near strands by field
cycling NMR relaxometry. It is a way to follow dynamical correlation from 1 ns to 10 μs.
We have shown that the Brownian relocation step is specific of the strand shape.
Implication in understanding intermittence Brownian dynamics over DNA (where an
unexpected $-\ln(\omega)$ is also observed [6] ) is undertaken. Influence of particle concentration
and particle conformation are also currently investigated.

Collaboration and acknowledgement: This work is performed in collaboration with D.
Constantin and P. Davidson from LPS, University of Orsay and M. Zinsmeister from
MAPMO, University of Orleans. France.

References
(2004)
Tracer Diffusion in HEMA Based Polymer Hydrogels

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1. Introduction
Dependence of diffusion coefficients characterizing mutual diffusion and self-diffusion of oligomeric tracers on the composition of equilibrium swollen polymer hydrogels was studied. The hydrogels were characterized by their dynamic correlation lengths $\xi$ measured by DLS and by concentrations of elastically active network chains $\nu$ determined by measurement of their elastic moduli. Diffusion coefficients of macroscopic translational diffusion of paramagnetic tracers (poly(ethylene glycol)s spin-labeled with nitroxides) in gels were measured by electron spin resonance imaging (ESRI) [1], self-diffusion and mutual diffusion coefficients of corresponding diamagnetic tracers (poly(ethylene glycol)s without nitroxides) were measured by pulsed-gradient stimulated NMR spin-echo (PGSTE) and dynamic light scattering (DLS), respectively.

2. Experimental
Hydrogels A, B, C, and D equilibrium-swollen with water were prepared by light-induced copolymerization of 2-hydroxyethyl methacrylate (HEMA) with 2-(2-hydroxyethoxy)ethyl methacrylate (DEGMA) using 2,3-dihydroxybutane-1,4-diyldimethacrylate as crosslinker and 2-hydroxy-2-methyl-1-phenylpropan-1-one as initiator. Compositions of batches leading to equilibrium-swollen hydrogels given in the Table were selected in preliminary experiments. Copolymerization was performed in sealed sample tubes the diameter of which was designed to meet demands of particular experiments: thin glass capillary of o.d. $\approx$ 1 mm for ESRI, glass sample tube of o.d. 4 mm for PGSTE NMR and glass sample tube of o.d. 10 mm for DLS, gravimetry and measurement of elastic moduli. Possible differences between the water contents in the batch and equilibrium water contents in the hydrogels were checked gravimetrically in long-term swelling experiments.

ESRI experiment was started by topping the hydrogel in the glass capillary (o.d. $\approx$ 1 mm, gel cylinder length 3 - 5 mm) with a drop of aqueous solution of the tracer (0.2 $\mu$L, $10^{-2}$ mol/L), the capillary was resealed and placed in the spectrometer cavity with the cylinder axis oriented vertically, parallel to the direction of the magnetic field gradient. Shapes of concentration profiles of the paramagnetic tracer inside the sample along the magnetic field gradient, approaching equilibrium distribution due to the tracer diffusion, were measured by ESRI [2] at suitable times after the diffusion start.

Diffusion coefficients of the paramagnetic tracers were found by analysis of the time dependence of the tracer concentration profiles, based on the solution of Fick’s equation appropriate for the sample geometry [3]. Diffusion coefficients of the diamagnetic...
derivatives of the tracers added to the polymerization mixture before polymerization were determined using PGSTE NMR and DLS.

3. Results

Data presented in the Table clearly show that the concentration of elastically active network chains \(v\) increases and dynamic correlation length \(\xi\) decreases with increasing concentration of the crosslinker in gels. In addition to the expected dependence of the diffusion coefficients of the tracers on their molecular weight and on the polymer concentration in the gel shown in the Figure, the experiments revealed their dependence on the crosslinker concentration (concentration of elastically active network chains). The differences found in diffusion coefficients measured in gel D by ESRI and QELS compared with the coefficients measured by PGSTE indicate that these techniques measure different diffusion processes.

4. Conclusion

The presented data indicate complexity of diffusion processes in polymer gels. Elucidation of the dependence of the diffusion coefficients on the gel composition requires more experimental data measured by various techniques in well characterized gel matrices covering a broader range of polymer fractions, crosslinking densities, and ratios of permanent and temporary crosslinks.

Acknowledgement. This research was supported by the Grant Agency of the Academy of Sciences of the Czech Republic (project A4050306). We thank P. Kadlec for performing light scattering measurements and M. Hlavičková (both IMC Prague) for preparation of gel samples.

References

Dynamic Crossover in Polymers, Role of Molecular Weight

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1. Introduction

One of the most characteristic features of glass-forming liquids is the sharp slowing down of relaxation times, \(\tau\), upon cooling. In these materials relaxation dynamics exhibit a non-Arrhenius temperature dependence usually described by Vogel-Fulcher-Tamman (VFT) relation \([1]\). However, for majority of glassforming liquids it is impossible to fit data in the entire temperature range with a single set of VFT parameters. Above some characteristic temperature it is necessary to use another set of VFT parameters. Existence of this dynamic crossover was predicted by the mode-coupling theory (MCT) \([2]\). The theory predicts a transition from a liquidlike to a solidlike dynamics on a molecular time and length scale at some specific temperature, \(T_C\), above the glass transition temperature \(T_g\). The dynamic crossover becomes even more evident with the use of derivative analysis proposed by Stickel \textit{et al} \([3]\), where \(x\) is the relaxation time or frequency. If \(\tau\) follows the VFT equation,

\[
\tau = \frac{T_B}{\phi_T} \left( \frac{dT}{dT} \right)^{1/2},
\]

where \(x\) is the relaxation time or frequency. If \(\phi_T\) should vary linear with \(T\). This is the so-called Stickel plot. In this plot, a change in the dynamics appears as a break in the linear dependence and the crossover temperature, \(T_B\), can be clearly identified. In most cases \(T_C > T_B\). Novikov and Sokolov check literature data for existence of crossover phenomena in different materials \([4]\). They found that \(T_B\) strongly depends on material whereas the relaxation time at the crossover temperature, \(\tau_c\), seems to have a rather universal value, \(\tau_c \sim 10^{-7} - 10^{-9}\) sec, for the majority of materials.

An important feature of supercooled liquids is also degree of departure of relaxation times from Arrhenius temperature dependence. Angell proposed classification of the liquids according to this deviation from activation behavior. He used the slope of the temperature dependence of relaxation times, presented vs \(T/T_g\), to estimate the so called “steepness index” or “fragility”, \(m\) \([5]\). According to this classification parameter, \(m\) increases with increasing deviation from Arrhenius-like behavior. It has to be emphasized, that ratio \(T_B/T_g\), correlates with the fragility of the system: the lower value of the ratio, the higher is the fragility.

2. Dynamic crossover in polymers

Polymers present an interesting example: one can change properties of these materials by changing only the chain length with no change in chemical structure. For some polymers,
like siloxanes, change of molecular weight has weak influence on relaxation dynamics [6]. For another group of polymers such as Polystyrene (PS) increasing molecular weight leads to significant changes of $T_g$ and $m$ [7]. It has been shown that polymers also exhibit a kind of dynamic crossover. Moreover, it seems that temperature dependences of chain and segmental relaxation dynamics becomes different at $T < T_C$. However, there is no study of molecular weight dependence of $T_g$.

We address this problem by investigations of Polyisoprene (PIP) and PS with different molecular weights using broadband dielectric spectroscopy (DS) and depolarized light scattering (LS).

In both polymers a dynamic crossover has been identified at temperatures significantly above their $T_g$'s. Moreover, the crossover temperature estimated from Stickel plot, $T_B$, and critical temperature obtained from the MCT analysis, $T_C$, are similar. The results show that increasing molecular weight leads to increases in the crossover temperature.

It has to be emphasized that the relaxation time at which crossover is observed remains independent of molecular weight and similar in both polymers. It is almost the same universal relaxation time, $\tau \sim 10^{-7.1}$ sec that has been reported for most of the non-polymeric systems.

References
1. Introduction

One of the most important features of supercooled liquids is degree of departure of relaxation behaviour from an Arrhenius temperature dependence. Angell proposed classification of the liquids according to this deviation. According to his classification the liquids with Arrhenius like temperature behaviour are called “strong” whereas liquids with strongly non-Arrhenius behaviour are called “fragile”.

Bulk water can be supercooled below its melting temperature down to ~235K. Above this temperature water is one of the most fragile liquids. Unfortuantely, below this temperature it inevitably crystallizes. Bulk water can vitrify only by hyperquenching, at rates > $10^5$ K s$^{-1}$. On the basis of the differential scanning calorimetry data, Angell et al. suggest that temperature of the glass transition of bulk water is located somewhere in the temperature range 160-180K. However real glass transition never can be observed for bulk water due to rapid crystallization at 150K. A way to avoid crystallization is to study water confined in various host materials. Although confinements generally affect the structure and dynamic properties of water, it is nevertheless possible to relate supercooled bulk water to studies of water in severe enough confinement with suppressed crystallization. For example, water could be confined as water of protein hydration. It is believed that ~ 0.4 g. of water per g. of protein is sufficient to cover the whole protein surface and fully activate the protein functionality. However, this hydration level is not sufficient for water crystallization and dynamics of supercooled water could be examined.

Results of recent investigations seem to exhibit new features of supercooled water in confinement. Chen and coworkers showed for water in different confinements that so-called fragile-to-strong transition (FST) occurs at characteristic temperature $T_c$~220K [1]. Below this temperature water exhibits Arrhenius temperature dependence of relaxation times, $\tau$, or self-diffusion coefficient. This is very unexpected and controversial results. First of all, this kind of transition has not been observed in any other liquid. Second, results from dielectric spectroscopy (DS) experiments presented by Swenson et al. showed that at temperatures above the FST, shape of the structural relaxation peak is asymmetric (as in many other liquids) whereas below the FST peak becomes symmetric. Symmetrically stretched spectral shape as well as activation behavior of relaxation times are characteristic features of so-called secondary relaxation process. Swenson et al. suggested that splitting of the structural and secondary relaxation take place at this temperature and no FST exists in water [2]. Thus, water exhibits some interesting behavior and microscopic nature of the observed anomalies remains unclear.
2. Conductivity in Protein Hydration Water

We perform DS investigations of water confined in lysozyme protein powder. Hydration level was about 0.4 g of water per g of proteins. It is enough to fully activate the protein functionality and to avoid water crystallization. We estimated temperature dependence of conductivity, $\sigma$, in the hydrated protein powder. Conductivity originates from translation of small, mobile ions, usually present in liquids. The correlation between $\tau$ and $\sigma$ is often observed. Such correlation is discussed in terms of Debye-Stokes-Einstein (DSE) relation $\sigma \tau = \text{const}$, which expresses a relationship between translational motions of different entities and the viscosity of the liquid. However, it was reported that for some materials decoupling between $\sigma$ and $\tau$ was observed. In this case, the relation between these two quantities can be described by the so called fractional DSE relation (FDSE), $\sigma^{s} \tau = \text{const}$ where the exponent $s$ is less than 1. It means that $\sigma$ is less sensitive to temperature changes than $\tau$. Opposite behavior was never observed. As it is visible from Figure 1, for glycerol – lysozyme powder mixture (0.8 g of glycerol per g of protein) temperature dependence of $\sigma$ follows the common picture described above. However, for water of hydration situation is quite different. $\sigma$ exhibits normal temperature behavior in the whole temperature range while $\tau$ from Chen et al. experiments demonstrates sudden change in temperature dependence at $T \sim 220$K. This sudden change has been ascribed to FST. Smooth temperature behavior of $\sigma$ clearly contradicts to this interpretation. It is not possible that sharp change in the temperature dependence of the main structural relaxation will not be reflected in conductivity. The only possible explanation is that the measured $\tau$ at low temperatures presents some secondary relaxation process.

3. Conclusion

Lack of changes in temperature behaviour of $\sigma$ suggests that no FST occurs at $T \sim 220$K in water of protein hydration. Strange temperature behaviour of the relaxation time can be ascribed to splitting of structural and secondary relaxations at this temperature.

References
Anomalous and Apparently Anomalous Diffusion in the Area of Neurophysiology

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1. Introduction

In the central nervous system information is transmitted from neuron to neuron due to functional contacts, or synapses, where a chemical intermediary, or neurotransmitter, releases following electrical signals in presynaptic cells; its binding to surface receptors triggers an influx of ions into the postsynaptic cells causing the shift of membrane potential away from the resting state. Glutamate releases in majority of brain synapses. The glutamate concentration time course in the synaptic cleft is influenced markedly by the geometry of the space that surrounds the synapse and the properties of glutamate diffusion in this geometry. Intracellular signals that lead to regulation of cell processes are transmitted by a limited number of small molecules, which are called second messengers. Their diffusion ensures the spreading of the signal all over the cell. Ca\(^{2+}\) is a unique molecule that relays signals mediated by membrane potential changes to the cell interior. Furthermore, in response to the binding of glutamate with metabotropic glutamate receptors, inositol 1,4,5-triphosphate (IP\(_3\)) is generated that releases Ca\(^{2+}\) from the intracellular stores.

Recently first communications that neurotransmitters in the extracellular space and second messengers in the dendrites of neurons can undergo anomalous diffusion appeared [1, 2]. Earlier diffusion kernel with fractional dimension was used for approximation glutamate diffusion in calyx of Held synapses [3]. Diffusion of IP\(_3\) in the spiny dendrites was proven to occur owing to trapping of molecules in these structures [2]. Nevertheless the causes of anomalous diffusion of both IP\(_3\) in smooth dendrites, and glutamate in the extracellular medium are not evident. Can the diffusion of neurotransmitters and second messengers be only apparently anomalous?

2. Simulation of glutamate diffusion and uptake in the extracellular space with complex geometry and IP\(_3\) diffusion and degradation in smooth neuronal dendrites

Previously we have shown that glutamate diffusion in the cerebellar glomerulus, a structure where a mossy fiber (MF) terminal makes synapses with dendrites of granule cells (GrCs), was much better approximated by equation for fractional Brownian motion (FBM) than by normal diffusion equation and suggested anomalous diffusion of the neurotransmitter [1]. For some short period of time (up to 2 ms) this observation could be explained by normal diffusion of glutamate from a 2-dimensional (2D) cleft between the MF terminal and the surface of dendrites into a 3-dimensional (3D) porous medium with a low volume fraction. Some transitory region exists, where effective diffusion coefficient and dimensionality depend on t.
Nonlinear time-dependence of spatial variance can also arise from time-dependence loss of molecules owing to binding with immobile buffers, degradation, or diffusion into other dendrites if diffusion of species in these structures is considered, but in this case a power-law relationship between variance and time is not observed.

It was shown that IP$_3$ in smooth dendrites diffuses with anomalous exponent 4.5. Intracellular binding and degradation were suggested to be candidates for such behavior [2]. To investigate this possibility, we developed a model of synaptically evoked Ca$^{2+}$ elevations in smooth GrC dendrites. This model included the mechanisms of Ca$^{2+}$ influx, release and buffering. The rates of IP$_3$ conversion to IP$_4$ via 3-kinase and their [Ca$^{2+}$]$_i$ dependence and to IP$_2$ via 5-phosphatase in range of experimental measurements were tested. The time constant of IP$_3$ degradation was much slower than the time course of IP$_3$ diffusion and could not produce anomalous diffusion behavior in our model.

Overcrowding of molecules causes anomalous diffusion only if molecules are large and have dimensions of dextrans or proteins in spite of significant retardation of the diffusion of both small and large molecules. IP$_3$ is a sufficiently small molecule with MW<0.5 kDa. Which physical processes can produce anomalous diffusion of IP$_3$ in smooth dendrites still remains unclear.

The other question, which we asked was if binding of glutamate transporters that are responsible for glutamate uptake from extracellular medium, can produce apparent anomalous diffusion. In the glomerulus transporters are situated on glial membranes at distance about 1.5 μm from the surface of MF terminal. The glutamate concentration transients were numerically integrated using a finite-difference method in an idealized model of glomerulus morphology. In our previous model [1] glutamate uptake was modeled by introducing an absorbing boundary for the diffusion field. In this work transporters that possessed kinetic properties of the transporter subtype GLAST of Bergman glial cells were included explicitly. Only the latest phase of currents mediated by glutamate spillover from neighboring release sites was influenced. Thus glutamate uptake by distantly situated transporters could not account for apparently anomalous glutamate diffusion. Glutamate buffers are not known and their existence is doubtful. Attachment plaques between dendrites could be considered as the sites of glutamate trapping, but their role is still ambiguous.

3. Conclusion
The causes of anomalous character of IP$_3$ diffusion in the smooth dendrites and glutamate diffusion in the extracellular medium of the cerebellar glomerulus still are not understood. Anomalous diffusion should be distinguished from the processes that resemble it. Our simulations show that IP$_3$ degradation or glutamate uptake by transporters could not produce anomalous diffusion behavior.

References
Anisotropic Diffusion of Flexible Random-Coil Polymers Measured in Brain Extracellular Space by Integrative Optical Imaging

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1. Introduction
In brain regions containing numerous parallel fibers, extracellular diffusion is anisotropic, i.e. faster along the fibers than across them, implying a preferential pathway for the transport of molecules in the extracellular space (ECS) [1,2]. To date, anisotropic diffusion in the ECS was measured with real-time iontophoretic method employing the small ion, tetramethylammonium (MW 74) [1,3,4] or with a FRAP-based technique using fluorophore-labeled dextran (MW 70,000) [5]. However, the ECS accommodates the diffusion of many endogenous macromolecules, and it is the exclusive route for interstitial transport of polymer-based drug carriers, therapeutic proteins, and virus-enclosed genes. To study how polymers of different sizes behave in an anisotropic ECS, we used integrative optical imaging (IOI) [6] to measure the diffusion of flexible random-coil dextran polymers (MW 3,000-525,000) in the molecular layer (ML) of the isolated turtle cerebellum (Fig. 1).

2. Methods and Results
The application of IOI to an anisotropic brain region was first validated using the small fluorophore Alexa Fluor 488 (MW 547). The fluorophore was pressure injected into the ML from a glass micropipette (Fig. 1) and a time series of images taken. The series was analyzed to calculate the effective diffusion coefficient ($D^*$, cm$^2$ s$^{-1}$) for major and minor axes of the elliptical 2-dimensional (2-d) projection of the 3-d diffusion cloud (Fig. 2). The diffusion anisotropic ratio (DAR=$D^*_{\text{major}}/D^*_{\text{minor}}$) was calculated. The measurements were then repeated using fluorophore-labeled dextran polymers (MW 3,000, 75,000, 282,000, 525,000). We found that $D^*$ decreased for AF488→dex525
(Table 1). The DAR increased for AF488, dex3, and dex75 but reached a plateau value at 1.78 for dex75, dex282, and dex525 (Fig. 3).

<table>
<thead>
<tr>
<th>Molecule</th>
<th>MW (kDa)</th>
<th>Agar gel</th>
<th>Molecular layer</th>
<th>DAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alexa Fluor 488</td>
<td>0.547</td>
<td>43.62 ± 2.71 (28)</td>
<td>1.1</td>
<td>21.23 ± 1.15 (12)</td>
</tr>
<tr>
<td>dextran (dex3)</td>
<td>3</td>
<td>23.25 ± 1.46 (20)</td>
<td>2.1</td>
<td>8.95 ± 1.31 (32)</td>
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<tr>
<td>dextran (dex75)</td>
<td>75</td>
<td>4.16 ± 0.16 (11)</td>
<td>11.9</td>
<td>1.24 ± 0.11 (10)</td>
</tr>
<tr>
<td>dextran (dex282)</td>
<td>282</td>
<td>2.34 ± 0.13 (10)</td>
<td>20.9</td>
<td>0.49 ± 0.09 (13)</td>
</tr>
<tr>
<td>dextran (dex525)</td>
<td>525</td>
<td>1.54 ± 0.09 (11)</td>
<td>31.8</td>
<td>0.23 ± 0.05 (9)</td>
</tr>
</tbody>
</table>

Table 1. Diffusion of Alexa Fluor 488 and dextran polymers in free media (Agar gel) and anisotropic ML (Molecular layer). Free diffusion coefficients (D) obtained in agar gel were used to calculate hydrodynamic diameters of molecules (Stokes-Einstein equation \( d_H = RT/3\pi\eta D \)). Data are expressed as means ± SD; number of measurements are in the parentheses; \( D \) and \( D^* \) values are at 25°C.

3. Conclusion
As in previous applications of the IOI method in isotropic brain regions [6,7], \( D^* \) decreased as the size of the dextrans increased. Surprisingly, the DAR reached a plateau for dex75, dex282, and dex525. This finding contrasts with results from modeling the diffusion of hard spheres between parallel rods, which predicts a monotonic rise in DAR with molecular size [8]. We hypothesize that the two largest dextran polymers approach the dimensions of the ECS, compelling them to deform from a spherical shape in order to traverse the narrow spaces; in fact, large dextran polymers are known to diffuse through pores that are smaller than their diameter [9]. Our findings have implication for the transport of endogenous macromolecules in the ECS and for the design of effective drug carriers.