diffusion-fundamentals.org
The Open-Access Journal for the Basic Principles of Diffusion Theory, Experiment and Application

Diffusion Properties and Models of Brain Interstitial Space

Charles Nicholson[1, 2]

[1]NYU Langone Health, New York, USA
[2]SUNY Downstate Health Sciences University, USA
E-Mail address: charles.nicholson@nyu.edu

(Received: 2025/10/06, Published online: 2025/11/03)

Introduction. The interstitial space (ISS) of the brain (often called the extracellular space) comprises the narrow channels that separate brain cells. The ISS is filled with a salt solution similar in composition to cerebrospinal fluid and a matrix of glycosaminoglycans and glycoproteins. The ISS allows the diffusion of ions and neuroactive molecules in the milieu surrounding brain cells and is crucial for maintaining electrical and chemical communication in the brain and for the delivery of drugs [1, 2 ,3]. Much of the ISS is only a few tens of nanometers wide and may be visualized as filamentous sheets. Recent studies, however, using a variety of imaging techniques have shown that the sheets of the ISS are punctuated by much larger voids [4].

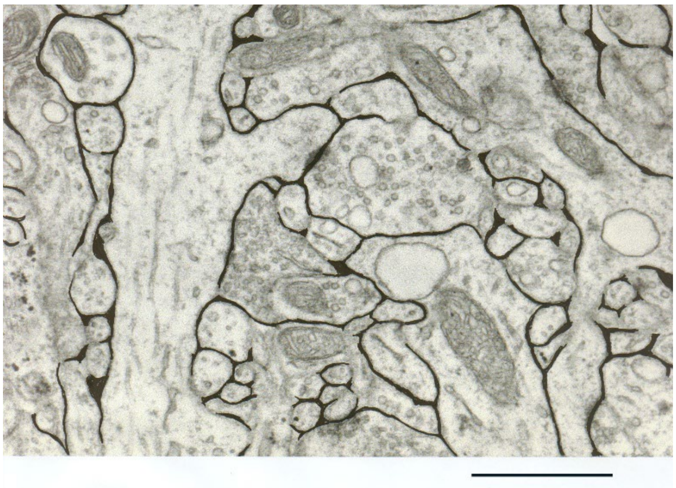

Fig. 1. Electron micrograph of a small region of the cerebral cortex of a rat. The black regions between cellular structures indicate the ISS, which may have been reduced in width by the histological processing. The scale bar represents 1 μm. Micrograph kindly supplied by Dr C B Jaeger. From [5].

Understanding diffusion in the ISS requires precise diffusion measurements using carefully chosen molecular probe molecules. Such data can be combined with structural information and theoretical models to gain insight. This brief communication will focus on two models.

Structural parameters of the ISS. The geometry of the ISS may be captured in two parameters: the porosity, or interstitial volume fraction, ϕ, and the geometrical tortuosity, τ_g. In a Representative Elementary Volume (REV) of brain tissue, the porosity, ϕ, is defined by:

$$\phi = \frac{\text{Volume of ISS in REV}}{\text{Volume of REV}}\,. \tag{1}$$

Geometrical tortuosity is a measure of the hindrance imposed on a diffusing molecule by the structure of the ISS and is defined here as:

$$\tau_g = \sqrt{\frac{D}{D^*}}$$

where D is the free diffusivity of a molecule (i.e. that is measured in water or dilute agarose gel) and D^* is the effective diffusivity measured in brain tissue when the molecule is confined to the ISS. In simulating diffusion in the ISS, it is assumed that the molecule executes a random walk and may undergo 'specular reflection' from the 'wall' of the ISS but otherwise does not interact with it. Geometric tortuosity may be thought of as a relative average path-length in the ISS.

diffus. fundam, 39 (2025) 1268, DOI: https://doi.org/10.62721/diffusion-fundamentals.39.1268

Measuring diffusion parameters. The Real-Time Iontophoretic (RTI) method [6] measures porosity and tortuosity by releasing tetramethyl-ammonium (TMA) cations from a micropipette and measuring the time-dependent concentration about 100 μm away using an ion-selective microelectrode (ISM), as shown in Fig. 2 [6]. Despite the diminutive size of the pore spaces, this method has revealed that the porosity ϕ of the ISS is about 20% of total brain tissue volume, i.e. $\phi = 0.2$. The tortuosity is typically ~1. 6 [7].

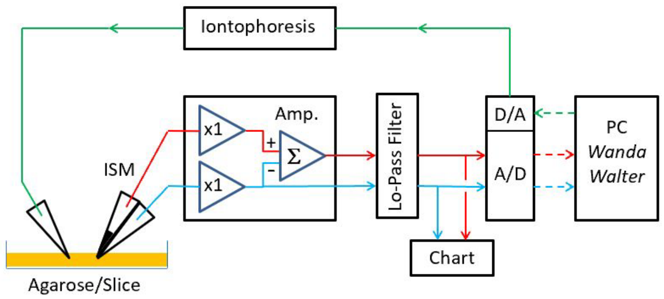

Fig. 2. Experimental setup for RTI method of measuring diffusivity. Measurements made in agarose (D) or brain slice (D^*). TMA is released from a micropipette by iontophoresis and the concentration registered about 100 μm away with an ion-selective microelectrode (ISM). Data analyzed using custom MATLAB programs Wanda and Walter. Note that the same method can be used in vivo. See [6] for details.

Models of the ISS. The experimental results were initially modeled by regarding the brain as an ensemble of cubic cells separated by thin sheets of ISS. It was established that this geometry generates $\tau_g \leq \sqrt{3/2}$ [8, 9], which is significantly less than the experimentally measured value of $\tau \sim 1.6$. So better models were required.

Corner Cubic Void Model. Taking account of the frequent voids in the ISS, a new model was developed called the Corner Cubic Void (CCV) model [10]. Here cubic cells of side $2a$ were modified with cubic voids of side b at each corner. Cells were separated by sheets of interstitial space of width $2w$ and the packed cells formed composite voids of width $2b$, as shown in Fig. 3.

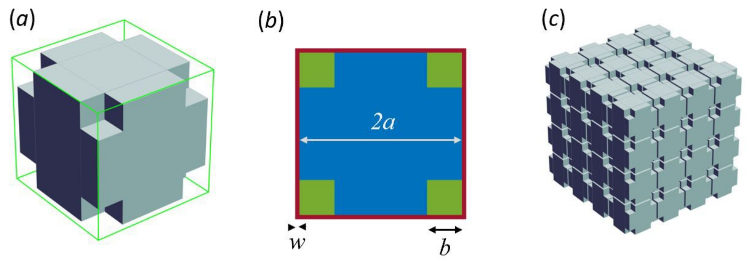

Fig. 3. Model of brain tissue. (a) unit cell with cubic corner voids. (b) top view of cell (c) ensemble of cells spaced $2w$ apart. In practice, 32^3 or 64^3 cells used for Monte Carlo simulations [10].

To realize the model, a value for the porosity ϕ is chosen considering the ISS in both the sheets and the voids. Typically, w is chosen at 20 nm and then a obtained in μm. The geometry is then scripted in the model description language associated with the MCell Version 3.4 software package [11, 12]. Point molecules, typically numbering 5,000 – 25,000 are released in the ISS at the origin of the model and allowed to execute random walks in the pore space. The effective diffusivity D^* is calculated as $D^* = (< r^2 >)/6t$ where r is the radial distance of each molecule from the origin at time t from release. As t increases, D^* reaches a steady-state value and at this time the molecules fit a Gaussian distribution in space and then $\tau = \sqrt{D/D^*}$ where D is the free diffusivity of the molecules.

Using the CCV model, it was found that, to obtain the experimental $\phi = 0.2$ and $\tau = 1.6$, the required geometry was $a = 0.74$ μm, $b = 0.38$ μm and $w = 20$ nm [10]. The presence of voids was essential to obtain the measured tortuosity and this feature was consistent with freeze-fixed electron microscopy and super-resolution optical imaging [4, 10]. Voids cause an increase in τ because they delay the overall transit of a diffusing molecule.

Random Cubic Void Model. The CCV model relied on a regularly repeating geometry, a constraint that is absent in normal brain tissue. A more realistic model, the Random Cubic Void (RCV) model, is under development where the voids are randomly distributed. The diffusion characteristics of the model are again being explored using Monte Carlo simulation of random walks in the ISS. The RCV model begins with a uniform set of cubic cells separated by narrow sheets of ISS. Cells are then randomly removed and this creates local voids in the ISS.

The present model consists of a base cluster of $Q = M \times M \times M$ cubic cells from which N cells are randomly removed (Fig. 4 (*a*)). To complete the model, the base cluster is replicated in all three x, y, z axes in the same way the original cubic cells were replicated in the base cluster (Fig. 4*(b)*). This leads to a total of $R = Q^2$ cells and $Q \times N$ voids. Replication is used because of the excessive computation burden of randomly removing cells in a cluster of size R. Values of $\phi = 0.2$, $M = 12$ and $2w = 40$ nm were chosen and Monte Carlo simulations run. To obtain $\tau = 1.6$ the value of a had to be 1.04 µm, so the width of a cube (and the void) was $2a = 2.08$ µm. Typical brain structures are not variations on a cube but may be approximately spherical, or take the form of elongated cylinders, branching trees or even be quite amorphous thus their extent is often greater than the 1 µm scale bar shown in Fig. 1. In the light of this, both the CCV and RCV models produce estimates of the size of the cells required to give the typical volume fraction and tortuosity that are consistent with the dimensions of cellular elements.

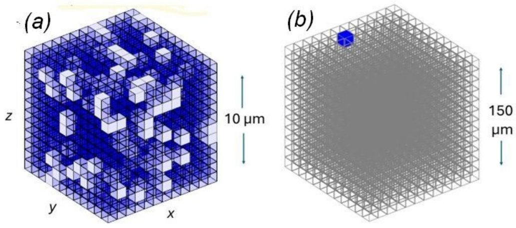

Fig. 4. Panel *(a)* shows a base cluster of cubic cells with $\phi = 0.2$ and $M = 12$ and the total number of cells in the base cluster is 1728 with missing (voids). The missing cells (white) are chosen at random. Each cell is 1.12 µm in width and the cells are separated by sheets of ISS $2w = 40$ nm wide. **Panel *(b)*.** The base cluster (blue cube in Panel *(b)*) is replicated and translated in three axes to arrive at a total of 2,985,984 cells spanning a width of 167 µm. Monte Carlo simulations of molecules executing random walks in the ISS pore space gave $\tau = 1.4$. (Nicholson)

Dynamic Properties of ISS

The ISS maintains constant structure over long periods. This is essential because it acts as a reservoir for the ions mediating electrical communication and as a conduit for chemical signals between cells. There are dynamic changes under some conditions, however. These fall into two types: diurnal variation during the sleep-wake cycle and pathophysiological changes.

Sleep-Wake Cycle. This brief survey has taken values of $\phi = 0.2$ and $\tau = 1.6$ as the normal values and these are indeed the values seen in most experiments where the animal is anesthetized or a brain slice is used. A study in 2013 [13] showed, however, that these values are the 'sleeping' values and that in an awake animal $\phi \sim 0.14$, although τ does not change. This study implies that the normal ISS is has a porosity of about 15% and this expands during sleep to around 20%. The study postulated that the increased ISS volume facilitates the removal of waste products during sleep by means of advection, i.e. bulk flow, in the ISS as part of a newly discovered glymphatic system. While diffusion in the ISS is generally accepted, advection in the ISS has been controversial for many years, mainly because there is no reliable method to measure it [14].

Ischemia and Stroke. When the blood supply to part, or all, of the brain is cutoff cells are deprived of oxygen and glucose, metabolism ceases and the cells swell, reducing the porosity and increasing geometrical hindrance to diffusion. The value of ϕ may drop to 0.05 and τ increase to 2.0 [7]. Restoration of the blood supply may reverse these changes if it occurs soon after the insult otherwise cell death ensues. Ischemia also causes major changes in the ionic content of the ISS, and all local electrical activity stops. When oxygen and glucose again become available energy driven molecular transporters in cell membranes restore the ionic content and the ISS porosity. Note that preparation of brain tissue for electron microcopy usually involves period of ischemia and consequently the porosity visible such micrographs (e.g. Fig. 1) show a reduced ISS width. Special cryo-fixation methods can preserve the ISS in limited brain regions [10].

Epileptic Seizure. Recent work has shown that epileptiform activity is accompanied by rapid volume transients in the ISS [15]. These transients appear to be mediated by a specific molecular transporter in brain cells and may play a role in the cascade of processes that drive epileptic events.

Conclusions. The ISS comprises the gaps between brain cells. It is very narrow and usually largely obliterated in classical electron micrographs. This brief review has focused on the use of diffusion studies with small molecules to produce models of the geometry of the ISS. The extracellular matrix present in the ISS may increase the local viscosity of the milieu and this has been considered in the modeling [10]. Other diffusion studies with macromolecules reveal a larger value of tortuosity that likely results from non-geometrical factors [1, 7, 16]. Overall, diffusion measurements in living tissue combined with appropriate models are revealing the true structure of the ISS and opening the way for a better understanding of chemical communication in the ISS and for improved drug delivery to the brain.

References

[1] C. Nicholson, S. Hrabětová, Brain extracellular space: The final frontier of neuroscience, Biophysical Journal, 113 (2017) 2133-2142.

[2] C. Nicholson, The secret world in the gaps between brain cells, Physics Today, 75 (2022) 26-32.

[3] C. Nicholson, Brain interstitial structure revealed through diffusive spread of molecules, in: A. Bunde, J. Caro, C. Chmelik, J. Kärger, G. Vogl (Eds.) Diffusive Spreading in Nature, Technology and Society, Springer, 2023, pp. 97-120.

[4] J. Tønnesen, S. Hrabětová, F.N. Soria, Local diffusion in the extracellular space of the brain, Neurobiology of Disease, 177 (2023) 105981.

[5] C. Nicholson, Diffusion and related transport properties in brain tissue, Reports on Progress in Physics, 64 (2001) 815-884.

[6] J. Odackal, R. Colbourn, N.J. Odackal, L. Tao, C. Nicholson, S. Hrabětová, Real-time iontophoresis with tetramethyl-ammonium to quantify volume fraction and tortuosity of brain extracellular space, Journal of Visualized Experiments (JoVE), (2017) e55755.

[7] E. Syková, C. Nicholson, Diffusion in brain extracellular space, Physiological Reviews, 88 (2008) 1277-1340.

[8] L. Tao, C. Nicholson, Maximum geometrical hindrance to diffusion in brain extracellular space surrounding uniformly spaced convex cells, Journal of Theoretical Biology, 229 (2004) 59-68.

[9] C. Nicholson, P. Kamali-Zare, Reduction of dimensionality in Monte Carlo simulation of diffusion in extracellular space surrounding cubic cells, Neurochemical Research 45 (2020) 42-52.

[10] C. Nicholson, Sheet and void porous media models for brain interstitial space, Journal of the Royal Society Interface, 20 (2023) 20230223.

[11] J.R. Stiles, T.M. Bartol, Monte Carlo methods for simulating realistic synaptic microphysiology using MCell, in: E. De Schutter (Ed.) Computational Neuroscience: Realistic Modeling for Experimentalists, CRC Press, London, 2001, pp. 87-127.

[12] www.mcell.org.

[13] L. Xie, H. Kang, Q. Xu, M.J. Chen, Y. Liao, M. Thiyagarajan, J. O'Donnell, D.J. Christensen, C. Nicholson, J.J. Iliff, T. Takano, R. Deane, M. Nedergaard, Sleep drives metabolite clearance from the adult brain, Science, 342 (2013) 373-377.

[14] T. Bohr, P.G. Hjorth, S.C. Holst, S. Hrabětová, V. Kiviniemi, T. Lilius, I. Lundgaard, K.A. Mardal, E.A. Martens, Y. Mori, U.V. Nägerl, C. Nicholson, A. Tannenbaum, J.H. Thomas, J. Tithof, H. Benveniste, J.J. Iliff, D.H. Kelley, M. Nedergaard, The glymphatic system: Current understanding and modeling, iScience, 25 (2022) 104987.

[15] R. Colbourn, J. Hrabe, C. Nicholson, M. Perkins, J.H. Goodman, S. Hrabětová, Rapid volume pulsation of the extracellular space coincides with epileptiform activity in mice and depends on the NBCe1 transporter, Journal of Physiology, 599 (2021) 3195-3220.

[16] R.G. Thorne, C. Nicholson, In vivo diffusion analysis with quantum dots and dextrans predicts the width of brain extracellular space, Proceedings of the National Academy of Sciences USA, 103 (2006) 5567-5572.