



Brief Communication

Determination of vascular permeability coefficients under slow luminal filling

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ARTICLE INFO

Article history:

Accepted 12 July 2013

Available online 23 July 2013

ABSTRACT

This Communication describes a method to obtain the permeability product (permeability coefficient normalized by vascular dimensions) from time-lapse intensity data for which the introduction of labeled solute into the vasculature does not occur at a sharply defined time. This method has an error of ~10% across a wide range of filling times and noise levels, and is particularly well-suited for situations in which the permeability coefficient is greater than 10^{-6} cm/s. We show that it is applicable whether the increase in vascular solute concentration is sustained or transient.

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Introduction

Quantitative measurement of solute permeability remains an important technique for assessment of microvascular physiology (Curry et al., 1983; Michel and Curry, 1999). The standard output in a permeability assay is the effective solute permeability coefficient P_e , which serves as a proportionality constant that relates the transvascular solute transport rate J_s , vascular surface area S_v , and plasma–tissue concentration difference ΔC (Curry, 1984):

$$J_s = P_e S_v \Delta C. \quad (1)$$

In a typical permeability assay, a labeled (e.g., fluorescent) solute is introduced abruptly into the vascular space at a concentration that is held constant thereafter. As solute begins to escape into the tissue, how quickly the total intensity $I(t)$, integrated over a region-of-interest that includes the vessel, increases with time yields the permeability coefficient multiplied by geometric factors (Huxley et al., 1987):

$$\frac{P_e S_v}{V_v} = \frac{1}{I_0} \frac{dI}{dt} \quad (2)$$

where I_0 refers to the instantaneous increase in intensity when the solute is introduced, and V_v refers to the vascular volume. This analysis assumes that the detected signal is proportional to solute concentration, and that the concentration of solute within the tissue is negligible compared to that in the vessel. For a single cylindrical vessel of radius r , Eq. (2) reduces to the widely-used relation $P_e = (1/I_0)(dI/dt)(r/2)$.

In many situations, however, it is impractical and/or impossible to introduce labeled solute to the observed vessels at a sharply defined

time. For instance, when mapping vascular permeability in vivo with magnetic resonance imaging (MRI) or computed tomography (CT), the contrast agent is injected at a point far from the vessels of interest (Schneider et al., 2011). Similarly, when using engineered vessels as models of microvascular physiology in microfluidic devices, the solute can be introduced substantially upstream of the vessel inlet (Chrobak et al., 2006). In these cases, the solute will disperse axially as it travels from the site of injection to the region that will be imaged (Calamante et al., 2000). As a result, Eq. (2) does not apply since I_0 is ill-defined and/or unmeasurable, and more sophisticated methods of determining permeability coefficients must be used. These methods typically require measurement of additional information, such as the vascular volume fraction in the region-of-interest, solute concentration within a large artery in the tissue, or solute dispersion coefficients (Hom et al., 2009; Sourbron and Buckley, 2012).

In this brief communication, we describe a numerical method to obtain the permeability product $P_e S_v/V_v$ solely from time-lapse intensity data without requiring instantaneous vascular filling. We demonstrate that this method is applicable to permeability assays that gradually lead to a plateau in the vascular concentration (“sustained infusion”) or that introduce a transient exposure to solute (“bolus injection”). Moreover, we use sensitivity analysis to determine how permeabilities, vascular filling times, bolus widths, and imaging noise levels affect the accuracy of this method.

Theory

When the imaging intensity is proportional to solute concentration, the following expression for the total intensity holds (Patlak et al., 1983):

$$\frac{dI}{dt} = \frac{dI_v}{dt} + \frac{P_e S_v}{V_v} I_v = \frac{dI_v}{dt} + \alpha_0 I_v \quad (3)$$

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where $\alpha_0 \equiv P_e S_V / V_V$. Here, I_V is the intensity due to solute that resides within the vessel lumen. After instantaneous filling, I_V is equal to I_0 in Eq. (2), and I is a linear function of time; for slow filling, I_V is generally not measurable. Eq. (3) can be inverted to yield:

$$I_V(t) = e^{-\alpha_0 t} \int_0^t e^{\alpha_0 t} \frac{dI}{dt} dt. \quad (4)$$

Although the permeability product α_0 is unknown, we can compute the right-hand-side of Eq. (4) from the measured intensity $I(t)$ for any guess α :

$$I_V(t; \alpha) = e^{-\alpha t} \int_0^t e^{\alpha t} \frac{dI}{dt} dt. \quad (5)$$

In practice, since the intensity I is only sampled at discrete times, the integral in Eq. (5) is approximated using standard numerical integration techniques (e.g., Simpson's rule).

The basis of our method is to vary the predicted permeability product α until the calculated vascular intensity $I_V(t; \alpha)$ matches what is known from the experimental protocol. For sustained infusion, we expect that the slope of $I_V(t; \alpha)$ at long times will reach a value of zero when α matches the true permeability product α_0 . For bolus injection, we expect that $I_V(t; \alpha)$ at long times will reach a value of zero when α equals α_0 .

Results and discussion

To illustrate this method of calculating permeability products, we first applied it to the ideal case where the vascular filling occurs

instantaneously at time $t = 0$ s and is sustained thereafter (Fig. 1A). In this case, the total intensity I increases linearly with time after filling (Fig. 1B), and straightforward integration of Eq. (5) yields:

$$I_V(t; \alpha) = \frac{\alpha_0}{\alpha} I_0 + I_0 \left(1 - \frac{\alpha_0}{\alpha}\right) e^{-\alpha t}; \quad t > 0. \quad (6)$$

Eq. (6) displays several important features (Fig. 1C): first, when the guess equals the true permeability (i.e., when $\alpha = \alpha_0$), the calculated vessel intensity I_V equals I_0 , the true vessel intensity after instantaneous filling; the slope of $I_V(t; \alpha_0)$ is thus zero. Second, when the guess overestimates the permeability ($\alpha > \alpha_0$), the slope of $I_V(t; \alpha)$ is negative. Third, when the guess underestimates the permeability, the slope of $I_V(t; \alpha)$ is positive.

We found that these same features also hold when vascular filling starts at $t = 0$ s but increases slowly (see Appendix for justification). Here, solute escapes substantially from the vessel before the vessel intensity has reached its maximum value. To test these ideas with intensity data that mimic the output of actual permeability assays, we generated model intensity data $I(t)$ for a chosen permeability product α_0 by assuming a vascular filling given by $I_V(t) = 1 - e^{-t^2/\tau^2}$, adding random noise, and sampling at a frequency of 10 Hz (Figs. 1D and E). We then used the model intensity data to calculate $I_V(t; \alpha)$ from Eq. (5) and determined the α that yielded a slope of zero, or a slope closest to zero, at the end of the time series (Fig. 1F). Surprisingly, even when the permeability product is large, the numerical method can still predict it with very good accuracy (Figs. 1G, H).

For bolus injection, the vascular intensity is expected to eventually approach zero. For the ideal case of a perfectly sharp and narrow bolus

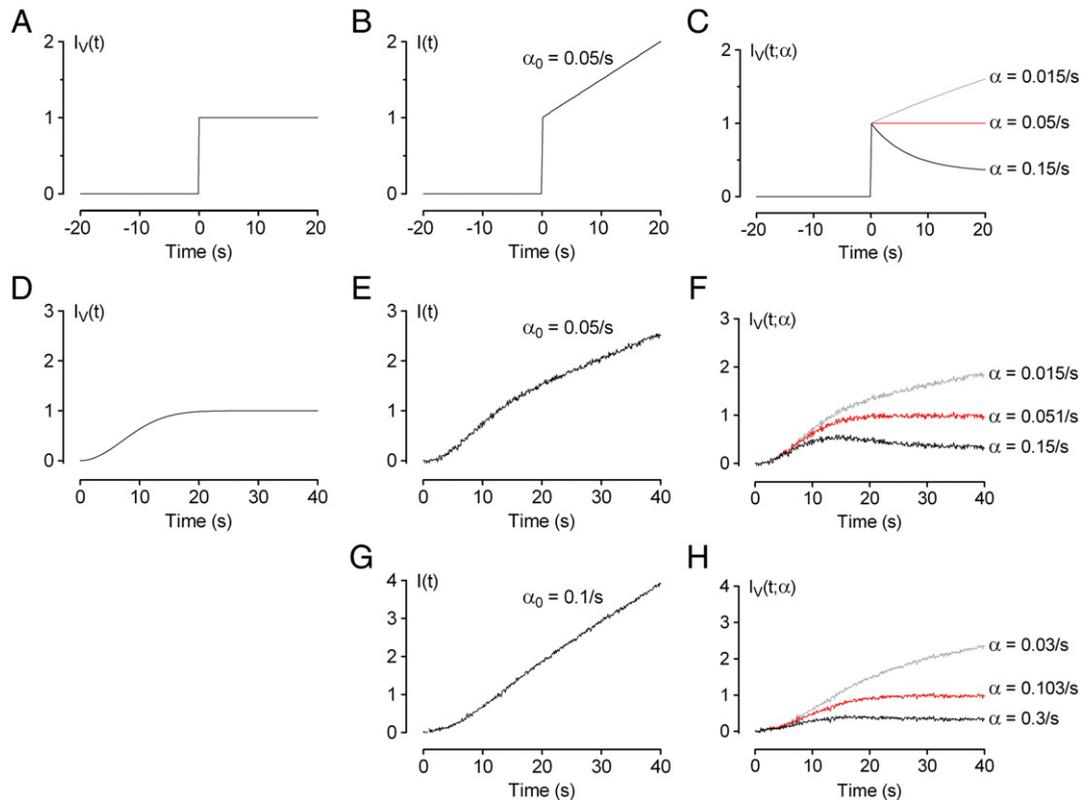


Fig. 1. Determination of permeability product from sustained infusion intensity curves. A) Vascular intensity $I_V(t)$ in arbitrary units for instantaneous filling. B) Total intensity $I(t)$ from (A) with $\alpha_0 = 0.05/s$. C) Calculated $I_V(t; \alpha)$ using (B). The slope of $I_V(t; \alpha)$ at long times equals zero when $\alpha = \alpha_0$. D) Vascular intensity $I_V(t)$ for non-instantaneous filling ($\tau = 10$ s). E) Total intensity $I(t)$ from (D) with $\alpha_0 = 0.05/s$ and 3% noise. F) Calculated $I_V(t; \alpha)$ using (E). The slope of $I_V(t; \alpha)$ at long times equals zero when $\alpha = 0.051/s$. G) Total intensity $I(t)$ from (D) with $\alpha_0 = 0.1/s$ and 3% noise. H) Calculated $I_V(t; \alpha)$ using (G). The slope of $I_V(t; \alpha)$ at long times equals zero when $\alpha = 0.103/s$.

that occurs at $t = 0$ s (Figs. 2A, B), Eq. (5) can be integrated exactly to yield:

$$I_V(t; \alpha) = N_0(\alpha_0 - \alpha)e^{-\alpha t} ; t > 0 \text{ s} \tag{7}$$

where N_0 is the area of the bolus (Fig. 2C). When the guess matches the true permeability product, $I_V(t; \alpha)$ equals zero. When the guess overestimates or underestimates the permeability, $I_V(t; \alpha)$ is negative or positive, respectively (Fig. 2C, inset).

Again, these features hold even when the bolus is not instantaneous. We generated model intensity data $I(t)$ by assuming a transient vascular concentration given by $I_V(t) = \frac{t^2}{\tau^2} e^{1-t^2/\tau^2}$ and adding random noise and discrete sampling (Figs. 2D, E). We then used the model intensity data to calculate $I_V(t; \alpha)$ and determined the α that yielded a value of zero, or a value closest to zero, at the end of the time series (Fig. 2F). As long as the bolus decayed to nearly zero by the end of the time series, the numerical method was able to predict the true permeability product accurately (Figs. 2G, H).

We performed a sensitivity analysis to determine the accuracy of the numerical method for ranges of permeability products ($\alpha_0 = 0.005\text{--}0.1/\text{s}$), filling times and bolus widths ($\tau = 5\text{--}15$ s), and noise

levels [1–5% of the maximum $I_V(t)$]. For each condition, we generated one hundred model intensity time series sampled at 10 Hz and lasting for forty seconds, used the methods described above to predict the permeability product, and calculated the average error. Tables 1 and 2 show the results for sustained infusion and bolus injection, respectively. For sustained infusion, we obtained the slope of $I_V(t; \alpha)$ at long times from a linear least-squares regression over the intervals $t = 15\text{--}40$ s, $25\text{--}40$ s, and $35\text{--}40$ s for $\tau = 5, 10, 15$ s, respectively. For bolus injection, we obtained the value of $I_V(t; \alpha)$ at long times by averaging the last ten points.

In general, the method performed extremely well, with average errors that were less than 10% for nearly all conditions. Higher noise and/or smaller permeability products reduced the performance, but even here, the errors were small. For instance, for a noise level of 5%, the method yielded an average error of ~8% for sustained infusion and ~10% for bolus injection. The largest error occurred for bolus injection when the permeability product was very small ($\alpha_0 = 0.005/\text{s}$), a condition for which our method would require a higher sampling rate to counteract the effects of signal noise.

We also tested the method with clinical CT time-intensity data [Table 3 in (Dankbaar et al., 2008)], in which bolus injection was

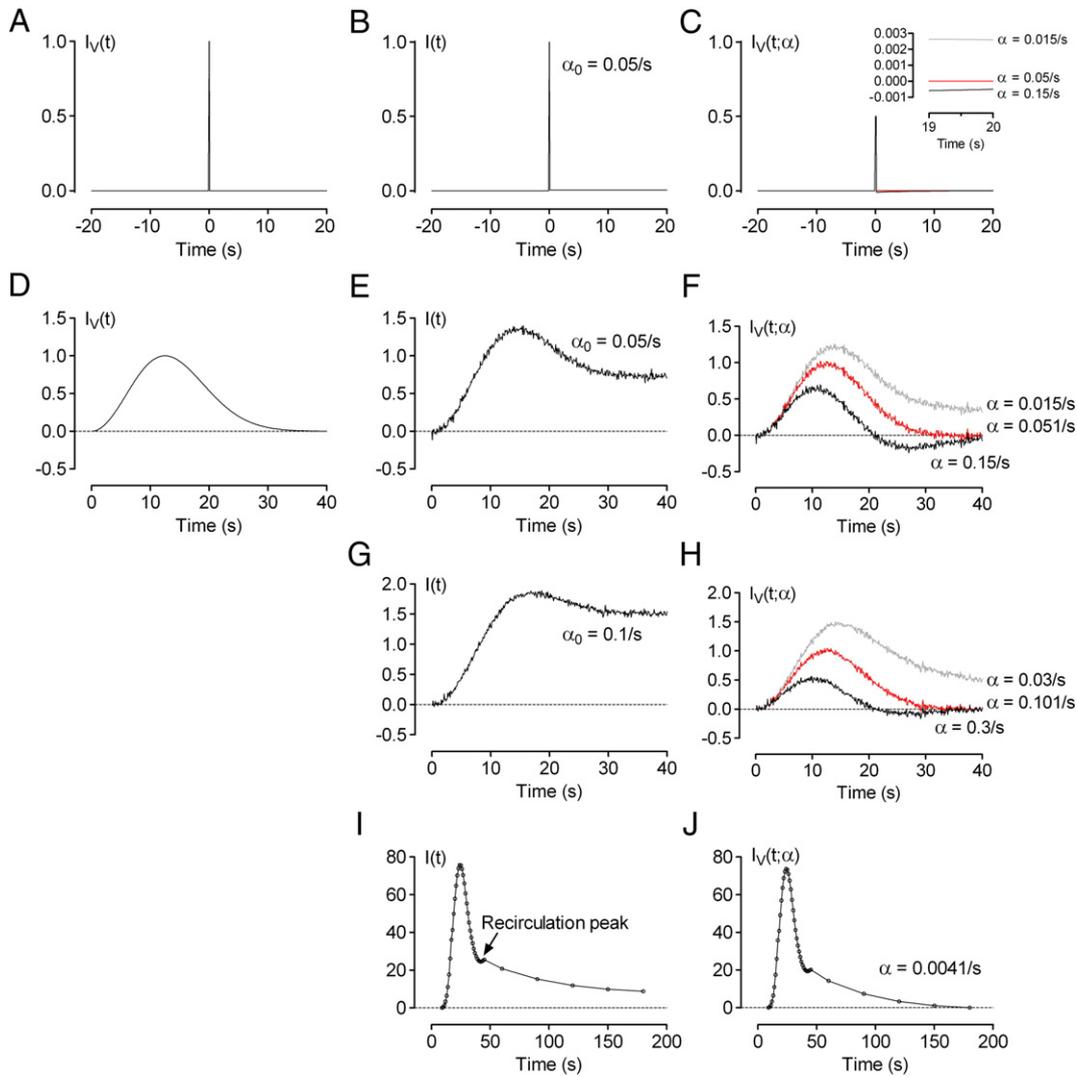


Fig. 2. Determination of permeability product from bolus injection intensity curves. A) Vascular intensity $I_V(t)$ for instantaneous bolus. B) Total intensity $I(t)$ from (A) with $\alpha_0 = 0.05/\text{s}$. C) Calculated $I_V(t; \alpha)$ using (B). Inset, $I_V(t; \alpha)$ equals zero when $\alpha = \alpha_0$. D) Vascular intensity $I_V(t)$ for a bolus of non-zero width ($\tau = 12.5$ s). E) Total intensity $I(t)$ from (D) with $\alpha_0 = 0.05/\text{s}$ and 3% noise. F) Calculated $I_V(t; \alpha)$ using (E). $I_V(t; \alpha)$ at long times equals zero when $\alpha = 0.051/\text{s}$. G) Total intensity $I(t)$ from (D) with $\alpha_0 = 0.1/\text{s}$ and 3% noise. H) Calculated $I_V(t; \alpha)$ using (G). $I_V(t; \alpha)$ at long times equals zero when $\alpha = 0.101/\text{s}$. I) Total CT image intensity [“tissue enhancement” from (Dankbaar et al., 2008)]. J) Calculated $I_V(t; \alpha)$ using (I). $I_V(t; \alpha)$ at long times equals zero when $\alpha = 0.0041/\text{s}$.

Table 1

Accuracy of the method for a sustained infusion modeled by $I_V(t) = 1 - e^{-t^2/\tau^2}$. Each row shows the predicted α (mean \pm SD) from one hundred noisy total intensity curves at which the calculated slope of $I_V(t; \alpha)$ was zero or closest to zero at long times.

α_0 (1/s)	Noise (%)	τ (s)	Predicted α (1/s)
0.05	3	10	0.050 \pm 0.002
0.005	3	10	0.0051 \pm 0.0006
0.1	3	10	0.107 \pm 0.005
0.05	1	10	0.0503 \pm 0.0007
0.05	5	10	0.051 \pm 0.004
0.05	3	5	0.050 \pm 0.001
0.05	3	15	0.054 \pm 0.013

followed by solute recirculation that decreased slowly (Fig. 2I). Because the sampling rate in the original dataset was lower than in our simulated datasets, we used the final time-point (rather than averaging the last ten) to obtain the value of $I_V(t; \alpha)$ at long times. The method predicted a permeability product of 0.0041/s, which agreed well with the value obtained from standard Patlak analysis (slope of ~ 0.03 /s and intercept of ~ 7 , yielding $\alpha \approx 0.0043$ /s), despite the presence of recirculation (Fig. 2J). In contrast to previous approaches, our method does not require knowledge of the intravascular solute concentration as a function of time.

The method described here allows one to determine the permeability product from an intensity time series without requiring instantaneous changes in vascular concentration. Because the equations used in current permeability assays often assume instantaneous vascular filling, the assays require elaborate experimental setups that are designed to minimize the time and distance between introduction of labeled solute and the imaging window. Our method only requires that the vascular intensity monotonically reaches a plateau or decays back to zero during the imaging time-window. The usefulness of the method is particularly apparent for solutes that have large permeability coefficients ($P_e > 10^{-6}$ cm/s, for which $P_e S_V/V_V$ is on the order of 0.01–0.1/s for capillaries) and/or when slow filling (on the order of several seconds) is unavoidable. For these cases, a substantial amount of solute may cross the vessel wall before the vascular concentration has reached a steady value.

In principle, it is possible to extend our method to more complex models of transvascular solute transport. For instance, one can account for the gradual extravascular accumulation of solute by modifying Eq. (3) (Sourbron and Buckley, 2012). The two ideas that underlie the method – estimation of the vascular intensity from measured total intensities and a guess of permeability, and evaluation of the vascular intensity on the basis of a known characteristic of the vascular filling curve – should hold regardless of the exact model used.

Acknowledgments

This work was supported by the National Heart, Lung, and Blood Institute (award number HL092335).

Table 2

Accuracy of the method for a bolus injection modeled by $I_V(t) = \frac{t^2}{\tau^2} e^{1-t^2/\tau^2}$. Each row shows the predicted α (mean \pm SD) from one hundred noisy total intensity curves at which the value of $I_V(t; \alpha)$ was zero or closest to zero at long times.

α_0 (1/s)	Noise (%)	τ (s)	Predicted α (1/s)
0.05	3	12.5	0.050 \pm 0.002
0.005	3	12.5	0.005 \pm 0.002
0.1	3	12.5	0.102 \pm 0.007
0.05	1	12.5	0.0503 \pm 0.0008
0.05	5	12.5	0.051 \pm 0.005
0.05	3	10	0.051 \pm 0.003
0.05	3	15	0.053 \pm 0.002

Appendix

Here, we provide the mathematical justification for the method of calculating permeability from an intensity time series. The derivation addresses the case of gradual filling (“sustained infusion”) that begins at $t = 0$ (i.e., $I_V = 0$ at $t = 0$) and ends at $t = T$, when the vascular intensity reaches a steady value (i.e., $dI_V/dt = 0$ at $t = T$). From Eqs. (3) and (5), we obtain:

$$\begin{aligned} \frac{\partial I_V(t; \alpha)}{\partial t} &= \frac{dI}{dt} - \alpha e^{-\alpha t} \int_0^t e^{\alpha t} \frac{dI}{dt} dt \\ &= \frac{dI_V}{dt} + \alpha_0 I_V - \alpha e^{-\alpha t} \int_0^t e^{\alpha t} \frac{dI}{dt} dt. \end{aligned} \quad (8)$$

Evaluation at $t = T$ and integration by parts yields:

$$\begin{aligned} \frac{\partial I_V(T; \alpha)}{\partial t} &= \alpha_0 I_V(T) - \alpha e^{-\alpha T} \int_0^T e^{\alpha t} \frac{dI}{dt} dt \\ &= \alpha_0 I_V(T) - \alpha e^{-\alpha T} \int_0^T e^{\alpha t} \left(\frac{dI_V}{dt} + \alpha_0 I_V \right) dt \\ &= (\alpha_0 - \alpha) \left[I_V(T) - \alpha e^{-\alpha T} \int_0^T e^{\alpha t} I_V(t) dt \right]. \end{aligned} \quad (9)$$

For a monotonically increasing vascular intensity, the term in brackets is positive since:

$$\alpha e^{-\alpha T} \int_0^T e^{\alpha t} I_V(t) dt < I_V(T) \alpha e^{-\alpha T} \int_0^T e^{\alpha t} dt < I_V(T). \quad (10)$$

In other words, the slope of $I_V(t; \alpha)$ at the end of the time series has the same sign as $\alpha_0 - \alpha$. When the guess α over- or underestimates the true α_0 , the slope of $I_V(t; \alpha)$ is negative or positive, respectively. Only when α equals α_0 is the slope zero.

For transient vascular filling (“bolus injection”), a similar analysis yields that over- or underestimation of the true α_0 causes the value of $I_V(t; \alpha)$ at long times to be negative or positive, respectively. Only when α equals α_0 is the value zero.

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