Articles in PresS. Am J Physiol Regul Integr Comp Physiol (February 23, 2011). doi:10.1152/ajpregu.00342.2010

Evaluation of Gravimetric Techniques to Estimate the Microvascular Filtration Coefficient

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RUNNING HEAD: Microvascular Filtration Coefficient Estimation

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ABSTRACT

Microvascular permeability to water is characterized by the microvascular filtration coefficient (K_f). Conventional gravimetric techniques to estimate K_f rely on data obtained from either transient or steady-state increases in organ weight in response to increases in microvascular pressure. Both techniques result in considerably different estimates and neither account for interstitial fluid storage and lymphatic return. We therefore developed a theoretical framework to evaluate K_f estimation techniques by 1) comparing conventional techniques to a novel technique that includes effects of interstitial fluid storage and lymphatic return, 2) evaluating the ability of conventional techniques to reproduce K_f from simulated gravimetric data generated by a realistic interstitial fluid balance model, 3) analyzing new data collected from rat intestine, and 4) analyzing previously-reported data. These approaches revealed that the steady-state gravimetric technique yields estimates that are not directly related to K_f, and are in some cases directly proportional to interstitial compliance. However the transient gravimetric technique yields accurate estimates in some organs, because the typical experimental duration minimizes the effects of interstitial fluid storage and lymphatic return. Furthermore, our analytical framework reveals that the supposed requirement of tying off all draining lymphatic vessels for the transient technique is unnecessary. Finally, our numerical simulations indicate that our comprehensive technique accurately reproduces the value of K_f in all organs, is not confounded by interstitial storage and lymphatic return, and provides corroboration of the estimate from the transient technique.

keywords: mathematical modeling, inverse problems, edema, Edemagenic Gain

INTRODUCTION

Central role of the microvascular filtration coefficient in edema formation. Interstitial fluid balance is determined by three processes—microvascular filtration, interstitial fluid storage and lymphatic return. Microvascular filtration determines the fluid flow into the interstitium; lymphatic return determines the fluid flow out of the interstitium. The difference between interstitial inflow and outflow leads to change in interstitial storage of fluid. The first process to be rigorously quantified, microvascular filtration, results from an imbalance of hydrostatic and colloid osmotic pressures, typically characterized by the Starling-Landis Equation (32, 35). The effects of these driving pressures are modulated by the permeability to water, characterized by the microvascular filtration coefficient (K_f). Some of the earliest interstitial fluid balance studies focused on developing techniques to estimate K_f of entire organs (18, 21, 22, 24, 28, 35, 41, 44), leading to vigorous debates as to which technique was superior (14, 31, 39).

Conventional techniques to estimate the microvascular filtration coefficient. Investigators have relied most extensively on gravimetric approaches based on the Starling-Landis Equation to estimate K_f by relating the rate change of interstitial fluid volume to the change in microvascular pressure (14, 31, 39). The two most widely used gravimetric techniques rely on similar experimental procedures to estimate K_f, but analyze the resulting data differently. With the *steady-state* gravimetric technique, the increase in interstitial fluid volume is determined by either measuring the steady-state increase in organ weight (15) or measuring the tissue “wet-to-dry” weight ratio (24) in response to microvascular pressure elevation. The rate of interstitial fluid accumulation is

determined by dividing the increase in interstitial fluid volume (compensated for vascular blood volume shifts) by the total time to reach steady state. K_f is estimated as the ratio of the rate of weight gain to the change in microvascular pressure. With the *transient* gravimetric technique, the weight gain is instead measured continuously. This additional information allows compensation for organ weight gain due to shifts in blood volume. After the initial period of rapid weight gain due to vascular engorgement, the rate of fluid accumulation is estimated either from the instantaneous rate of change in weight or the rate of change in weight extrapolated to the beginning of pressure elevation (22, 31, 39). Variation in selection of the time points for the calculation of the rate of change in weight results in disparity in K_f estimates; especially when using the instantaneous rate of change (37). The relative ease of gravimetric techniques to estimate K_f has led to their popularity, despite the evidence that they yield inconsistent estimates of K_f, even under the same experimental conditions (14, 31, 39).

Problems common to conventional gravimetric techniques. Because both analysis techniques to estimate K_f from gravimetric data use the same fundamental equation characterizing microvascular filtration, they share similar theoretical limitations. First, interstitial fluid pressure is assumed constant, and thus its effect on microvascular filtration is neglected (14, 39). However, increases in microvascular pressure have been demonstrated to raise interstitial fluid pressure, which acts to retard microvascular filtration (3). Second, the effect of interstitial fluid storage capacity, characterized by interstitial compliance (C), is neglected. It has been demonstrated that interstitial fluid volume is very sensitive to interstitial compliance (10). Third, organ weight gain is assumed to be a function of inflow only, and lymphatic return from the tissue,

characterized by the effective lymphatic resistance (R_L) (13), is neglected. However, it is the difference between interstitial inflow and outflow rates that determines the rate of organ weight gain (3). Dealing with these complexities has not been feasible, since until recently there has been no theoretical framework to evaluate the impact of interstitial fluid storage and lymphatic return on these estimation techniques. The concept of the "Edemagenic Gain" (10) provides just such a framework. Briefly, the sensitivity of a system to edemagenic conditions is characterized by the Edemagenic Gain, defined as the change in interstitial fluid volume resulting from a change in microvascular driving pressure. Assuming simple linear functions characterizing microvascular filtration, interstitial fluid storage, and lymphatic return, the Edemagenic Gain of an organ was shown to depend on K_f, as well as R_L and C (10). This concept thus yields a comprehensive theoretical model that allows prediction of transient and steady-state interstitial fluid volumes that incorporates the effects of interstitial fluid storage and lymphatic return. Therefore the purpose of the present work is to utilize the concept of Edemagenic Gain to develop a comprehensive K_f estimation technique and evaluate conventional K_f estimation techniques.

THEORY

Interstitial inflow of fluid. The Starling-Landis Equation (*Eq. 1*) characterizes the fluid filtration rate (J_V) across a microvascular membrane into the interstitium (32, 35).

$$J_V = K_f\left[P_c - P_i - \sigma\left(\Pi_c - \Pi_i\right)\right] \qquad (1)$$

The difference between microvascular (P_c) and interstitial (P_i) hydrostatic pressures tends to force fluid into the interstitium. The difference between microvascular (Π_c) and

interstitial (Π_i) colloid osmotic pressures tends to draw fluid in the opposite direction, from the interstitium into the microvessels. The reflection coefficient (σ) characterizes the relative permeability of the microvasculature to plasma proteins (having a value between 0 and 1), and thus modulates the contribution of colloid osmotic pressure to effective microvascular driving pressure. The microvascular filtration coefficient (K_f) depends on microvascular surface area and permeability to water, and modulates the effective microvascular driving pressure. Although originally derived for a single microvascular membrane, *Eq. 1* has come to be used to characterize the gross microvascular filtration rate of an entire organ (33, 35).

Interstitial outflow of fluid. The Drake-Laine Equation (*Eq. 2*) characterizes lymphatic function by relating lymph flow rate (J_L) to an effective lymphatic driving pressure (13).

$$J_L = \frac{\left(P_i + P_p - P_{out}\right)}{R_L} \qquad (2)$$

Since lymphatic outlet pressure (P_{out}) is typically greater than interstitial fluid pressure (P_i), the difference in P_i and P_{out} tends to retard lymph flow. The value of (P_i+P_p) represents the lymphatic driving pressure and is composed of interstitial hydrostatic pressure and lymphatic pumping pressure (P_p). The effective lymphatic resistance (R_L) is the slope of the relationship between effective lymphatic driving pressure (P_i+P_p-P_{out}) and the resulting lymph flow. In this formulation, P_p and R_L are empirically-derived parameters used to describe the lymphatic pressure-flow relationship.

Characterizing interstitial fluid storage. The interstitium's capacity to store fluid volume (V) is a fundamental mechanical property characterized by the "interstitial

compliance" (*C*), the slope of the interstitial fluid volume-pressure relationship (*Eq. 3*) (23, 38),

$$P_i = P_o + \frac{V}{C} \tag{3}$$

where P_o is an empirical constant. The relationship between interstitial fluid pressure and volume depends on tissue hydration level and can be highly nonlinear (23, 38). However, the interstitial fluid volume-pressure relationship has been approximated to be "piecewise linear," a common technique to deal with the effect of hydration on interstitial compliance (5, 6, 25).

Conservation of mass. Based on the principle of conservation of mass, the rate of change in interstitial fluid volume (*V*) is determined by the difference between the rates of interstitial inflow (J_V) and outflow (J_L) (*Eq. 4*).

$$\frac{dV}{dt} = J_V - J_L \tag{4}$$

In steady state, interstitial fluid volume ceases to change, and thus *dV*/*dt* equals zero. Therefore, inflow is balanced with outflow, and J_V=J_L.

Solution of the differential equation. Interstitial fluid volume is obtained by solving *Eq. 4* by standard techniques.

$$V(t) = \left(1 - e^{\frac{-t(1+R_L K_f)}{CR_L}}\right)\left\{\frac{CR_L K_f}{1+R_L K_f}\cdot\left[P_c - P_o - \sigma(\Pi_c - \Pi_i)\right] - \frac{C}{1+R_L K_f}\cdot\left(P_o + P_p - P_{out}\right)\right\} + V_{initial} \tag{5}$$

The change in interstitial fluid volume, ΔV, in response to a step change in microvascular pressure, $\Delta P_{c,}$ is obtained by calculating the difference between interstitial fluid volume

at initial and final microvascular pressures. Assuming that P_p, P_{out}, R_L, K_f, C, Π_c and Π_i do not change with increasing microvascular pressure (P_c), the change in interstitial fluid volume is given by *Eq. 6*,

$$\Delta V(t) = \left(1 - e^{\frac{-t}{\tau}}\right)\left[EG \cdot \Delta P_c\right] \tag{6}$$

where the steady-state Edemagenic Gain (*EG*) (10) is

$$EG = \frac{\Delta V}{\Delta P_c} = \frac{CR_L K_f}{1 + R_L K_f}, \tag{7}$$

and the Edemagenic Time Constant (τ) is

$$\tau = \frac{CR_L}{1 + R_L K_f} = \frac{EG}{K_f}. \tag{8}$$

Illustrative example of interstitial fluid volume changes in the sheep lung. Based on reported parameters characterizing the sheep lung (Table 1), the predicted change in interstitial fluid volume resulting from a step change in microvascular pressure is illustrated in Fig. 1. The Edemagenic Gain characterizes the steady-state change in interstitial fluid volume, whereas the Edemagenic Time Constant determines the rate at which interstitial fluid volume changes with change in P_c.

METHODS

Analytical evaluation

Conventional transient and steady-state gravimetric K_f estimation techniques. Conventional techniques to estimate K_f only utilize the Starling-Landis Equation (*Eq. 1*). In the case of the steady-state gravimetric technique, ΔJ_V is estimated by dividing the

total amount of fluid accumulated (ΔV) (approximated by the weight gain from initial to steady-state P_c) by the time to reach steady state (t_{ss}). In the case of the transient gravimetric technique, ΔJ_V is estimated by the change in interstitial fluid volume (ΔV) over a short period of time (Δt). With the transient technique, lymph flow is set to zero, typically by tying off the efferent lymphatic vessels (14). This corresponds to setting the effective lymphatic resistance to infinity.

Steady-State Gravimetric Technique $K_f = \frac{\Delta J_V}{\Delta P_c} \qquad \Delta J_V = \frac{\Delta V}{t_{ss}} \qquad (J_L \text{ neglected}) \qquad (9)$

Transient Gravimetric Technique $K_f = \frac{\Delta J_V}{\Delta P_c} \qquad \Delta J_V = \frac{\Delta V}{\Delta t} \qquad (R_L = \infty;\ J_L = 0) \qquad (10)$

Although both conventional gravimetric techniques are reported to estimate K_f using *Eq. 1*, how these estimates are altered by lymphatic return (*Eq. 2*) and interstitial fluid storage (*Eq. 3*) has not been quantified.

Novel comprehensive technique to estimate microvascular filtration coefficient using the Edemagenic Gain and the Edemagenic Time Constant. A simple and comprehensive technique to estimate K_f from both steady-state and transient gravimetric data arises from the equations characterizing changes in interstitial fluid volume. Solving *Eqs. 7* and *8* simultaneously for K_f results in a ratio of the Edemagenic Gain and the Edemagenic Time Constant (Fig. 1).

Comprehensive Gravimetric Technique $K_f = \frac{EG}{\tau} \qquad (11)$

Because EG is divided by τ, R_L and C cancel out (*Eq. 11*). Although interstitial storage and lymphatic return are not neglected, these processes do not affect the comprehensive

K_f estimation technique. Therefore, *Eq. 11* forms the basis of a novel K_f estimation technique that is independent of particular values of effective lymphatic resistance and interstitial compliance. Particular techniques that sever lymphatic vessels or remove restrictive membranes in the process of excising organs (thus inadvertently altering R_L and C) would thus have no impact on the estimate of K_f.

Numerical evaluation

Comparison of comprehensive and conventional K_f estimation techniques using simulated experiments. Because the transient technique does not require steady-state data, investigators using this technique typically do not report the time to steady state or the steady-state interstitial fluid volume. Similarly, because the steady-state technique does not require transient data, investigators typically do not report transient effects. Therefore, to compare our novel comprehensive technique with conventional K_f estimation techniques, we applied each technique to simulated experiments where the "true" value of K_f used to generate the simulated data was known. The complex mathematical model used to simulate experiments was introduced elsewhere (9) to illustrate how interstitial fluid volume changes with a rapid change in interstitial compliance. This complex model can be used to simulate both steady-state and transient changes in interstitial fluid volume. Furthermore, this model included variable microvascular fluid filtration and protein extravasation, storage of interstitial fluid and proteins, and lymphatic return of fluid and proteins. The assumption of a variable interstitial protein allows the model to capture the effect of “protein washdown”, a decrease in the interstitial protein concentration with increased microvascular fluid

filtration (40, 42). This complexity in particular allowed us to quantify the error in K_f estimates arising from the common assumption of constant interstitial protein concentration. We created three simulated experiments based on parameters from three different organs listed in Table 1. In each case, interstitial fluid volume was simulated in response to a 50% increase in microvascular pressure. Using transient and steady-state changes in interstitial fluid volume, K_f was estimated with three techniques: the steady-state gravimetric technique (*Eq. 9*), the transient gravimetric technique (*Eq. 10*), and the comprehensive technique (*Eq. 11*). In this case, the time to reach steady state was taken to be the time when tissue weight did not increase by more than 1mg/minute/100g. The error in K_f estimation was determined by comparing the estimated value ($K_{f\,estimated}$) with the value used to generate the simulated data ($K_{f\,assumed}$).

$$\%Error = \frac{K_{f\,assumed} - K_{f\,estimated}}{K_{f\,assumed}} \cdot 100 \qquad (12)$$

Illustration of how experimental changes in microvascular, lymphatic and interstitial parameters could affect K_f estimation. Experimentally, some pharmacological interventions under study, as well as surgical procedures such as organ excision, may alter not just microvascular filtration (K_f) but also lymphatic function (R_L), interstitial function (C), the microvascular reflection coefficient (σ) and the microvascular protein permeability-surface area product (PS). To determine whether the comprehensive and conventional gravimetric K_f estimation techniques are confounded by incidental alterations in R_L, C, σ and PS, the complex, realistic mathematical model reported previously (9) was used to simulate experiments based on sheep lung parameters (Table 1). True K_f was also compared to K_f estimated when there was a simulated 50% increase in R_L or C, or a 50% decrease in σ or PS. The alterations in R_L, C, σ and PS were

assumed to be made before P_c was elevated, and thus the values of R_L, C, σ and PS were assumed to be constant during the actual K_f estimation process.

Illustration of how errors in microvascular and interstitial variables could affect K_f estimation. To determine how errors in P_c, Π_c, and changes in Π_i affect K_f estimation using gravimetric techniques, the complex, realistic mathematical model reported previously (9) was used to simulate experiments based on sheep lung parameters (Table 1). When P_c, Π_c and Π_i are all measureable directly, *Eq. 7* can be generalized to include the effects of inadvertent changes in Π_c and Π_i on the Edemagenic Gain (10).

$$EG = \frac{\Delta V}{\Delta[P_c - \sigma(\Pi_c - \Pi_i)]} = \frac{CR_L K_f}{1 + R_L K_f}, \qquad (13)$$

This formulation combines critical variables (P_c, Π_c and Π_i) into one composite variable, the microvascular driving pressure [P_c-σ(Π_c - Π_i)]. This composite variable allows easy evaluation of the effect of particular measurement errors on K_f estimation. (1, 46) (9) This model was used to evaluate the effect of a 20% error in the microvascular driving pressure on K_f estimates. In this complex model, Π_i varies with microvascular filtration rate, allowing us to quantify how the assumption of a constant Π_i impacts K_f estimates (*Eq. 13*). The resulting value of K_f estimated from our comprehensive and conventional gravimetric techniques was compared to the true K_f.

Experimental evaluation

Surgical preparation. Experimental procedures and animal care were performed in compliance with protocols approved by the Texas A&M University Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (n=6, 313±10 g) were

fasted overnight with free access to water before surgery. Rats were anesthetized with inhaled isoflurane (5% induction, 2.5% maintenance). Rectal temperature was monitored with a thermistor probe and maintained at 37°C with a Homeothermic Controller and Plate (ADInstruments ML295/R). Systemic arterial pressure was monitored with a pressure transducer (ADInstruments MLT844) through a cannula placed in the left femoral artery. A midline abdominal incision was made, and a part of the small intestine was exteriorized. A segment with intact vasculature and mesentery was isolated from the rest of the intestine and was flushed with isotonic saline to remove residues from the lumen. With ends tied, the small intestinal segment was placed on a weighing dish and was covered with a plastic wrap. A loop of suture was placed around the superior mesenteric vein draining the small intestine and was used as a snare to manipulate venous pressure. One of the small veins from the intestinal segment close to mesentery was cannulated, and venous pressure was measured with a pressure transducer (ADInstruments MLT844) connected to the cannula. Finally, the weighing dish containing the covered intestinal segment was connected to a force transducer (ADInstruments FT03), and the rest of the intestine was covered with wet gauze soaked in isotonic saline. Systemic arterial pressure, mesenteric venous pressure and intestinal segment weight were continuously recorded at 3Hz using a data acquisition system (ADInstruments PowerLab 7) connected to a PC.

Experimental protocol. After recording the intestinal segment weight at baseline for 10 min, the snare around superior mesenteric vein was manipulated to increase the venous pressure by 20.51±0.6 cmH_2O. The change in microvascular pressure was estimated from the mesenteric venous pressure, and was taken to be 75% of the change in

mesenteric venous pressure, consistent with a previous study (8). This resulted in a 15.38±.43 cmH_2O increase in microvascular pressure. The resulting transient and steady-state changes in weight of the intestinal segment were recorded. The time to reach steady state was taken to be the time when tissue weight increased by less than 3mg/min/10g, within the resolution of our equipment in our experimental preparation.

K_f estimation using comprehensive, transient and steady-state gravimetric techniques. Using the rate of change, two phases (rapid and slow) were identified for the weight gain of the intestinal segment. To ensure that changes in organ weight were not due to vascular volume shifts as a result of engorgement, the initial rapid phase was neglected (11, 22, 31). The Edemagenic Gain was estimated by dividing the steady-state organ weight gain during the slow phase by the increase in microvascular pressure. The Edemagenic Time Constant was estimated from the slope of the regression line of the natural log of the weight gain as a function of time. Using the comprehensive technique, K_f was estimated by the ratio of the Edemagenic Gain and the Edemagenic Time Constant. Using the transient gravimetric technique, K_f was estimated by dividing the rate of increase in organ weight during slow phase extrapolated to the initial time (i.e., t =0) by the increase in microvascular pressure. Increase in organ weight during the 20 min time period after raising the microvascular pressure was analyzed to estimate K_f. The experimental period of 20 min was selected to minimize the contribution of vascular stress-relaxation, which typically is negligible after 7-9 min (37). Using the steady-state technique, K_f was estimated by the ratio of rate of change in organ weight to the increase in microvascular pressure. The rate of change in organ weight was determined by

dividing the steady-state increase in organ weight during the slow phase by the time to steady state.

Collection and analysis of previously reported gravimetric data. To further broaden the scope of the current work and illustrate the use of the three techniques to estimate K_f, experimental data (intact dog lung) previously reported by Drake et al. were analyzed (15). Briefly, dogs were anesthetized, the left chest was opened, and balloon-tipped catheters were placed in into the artery feeding the upper left lobe of the lung and the vein draining the lower left lobe. Pulmonary artery and lower left lobar venous pressures were manipulated with balloon-tipped catheters, and microvascular pressure (P_c) was calculated as the average of the pulmonary arterial pressure and the lower left lobar venous pressure. P_c was elevated in steps of 5 mmHg, and the resulting change in lung weight was recorded every minute and normalized with the weight of the normal lobe. Organ weight gain during the slow phase was used to estimate K_f using the comprehensive, transient and steady-state gravimetric techniques. The value of K_f originally reported by Drake et al. (13) was not used as a point of comparison, because their particular method required a large increase in microvascular pressure, which is reported to underestimate K_f (39).

RESULTS

Analytical evaluation

What do conventional techniques estimate? Substituting *Eqs. 6-8* into *Eq. 9* provides insight into what the conventional steady-state analysis of gravimetric data yields.

$$\frac{\Delta J_V}{\Delta P_c} = \frac{\Delta V / t_{ss}}{\Delta P_c} = \frac{EG}{t_{ss}} = \frac{1}{t_{ss}} \cdot \frac{CR_L K_f}{1 + R_L K_f} = \frac{\tau}{t_{ss}} \cdot K_f \qquad (14)$$

As steady state is reached, the exponential term in *Eq. 6* degenerates to zero. Thus, it is evident from *Eq. 14* that the steady-state gravimetric technique estimates the ratio of Edemagenic Gain to time required to reach steady state. This is notably not equal to K_f. Similarly, substituting *Eqs. 6-8* into *Eq. 10* provides insight into the parameters that determine the value of the conventional transient analysis of gravimetric data. Replacing the exponential term in *Eq. 6* by its Taylor series expansion (43) provides a convenient means to approximate the solution. In the case of the transient gravimetric technique, lymphatic vessels are tied off, resulting in very high values of R_L. Under this condition, the Edemagenic Gain is proportional to interstitial compliance (10) and Edemagenic Time Constant is proportional to C/K_f (*Eq. 8*).

$$\frac{\Delta J_V}{\Delta P_c} = \frac{\Delta V / \Delta t}{\Delta P_c} \cong \left(1 - e^{\frac{-t}{C/K_f}}\right) \cdot \frac{C}{\Delta t} \cong \left(\frac{\Delta t}{C/K_f} - \left(\frac{\Delta t}{C/K_f}\right)^2 + \ldots\right) \cdot \frac{C}{\Delta t} \cong K_f \qquad (15a)$$

When the experimental time period (Δt) is short (i.e., close to zero), the higher order terms in *Eq. 15a* can be neglected, and the transient gravimetric technique approaches K_f. Alternatively, *Eq. 15b* represents the case where lymphatic vessels leaving the organ are not tied.

$$\frac{\Delta J_V}{\Delta P_c} = \frac{\Delta V / \Delta t}{\Delta P_c} = \left(1 - e^{\frac{-t}{\tau}}\right) \cdot \frac{EG}{\Delta t} \cong \left(\frac{\Delta t}{\tau} - \left(\frac{\Delta t}{\tau}\right)^2 + \ldots\right) \cdot \frac{EG}{\Delta t} \cong K_f \qquad (15b)$$

In this case, neglecting the higher order terms also results in K_f, whether or not lymphatic vessels are tied to stop lymphatic drainage.

Numerical evaluation

Comparison of K_f estimation techniques. Table 1 lists the values of K_f estimated from the comprehensive technique, as well as those from transient and steady-state gravimetric techniques. The increases in interstitial fluid volume were obtained from the three simulated experiments (using parameters listed in Table 1) in response to 50% increase in microvascular pressure. The comprehensive technique estimates K_f with less than 9% error in all organs. Although the transient gravimetric technique estimates have similar small error, the error using the steady-state technique was considerable for all cases. Figure 2 illustrates the effect of increasing experimental time (Δt) on K_f estimation using the transient gravimetric technique in the three simulated experiments. When longer experimental times are used for determining the rate of change of organ weight, the higher order terms in *Eq. 15b* produce significant error in K_f estimated using the transient gravimetric technique.

Effect of change in R_L and C on K_f estimation. Figure 3 illustrates the effect of a simulated incidental increase in R_L and C on K_f estimates using the comprehensive, transient and steady-state gravimetric techniques. A 50% increase in effective lymphatic resistance led to a 16% increase in the Edemagenic Gain and a 13% increase in the Edemagenic Time Constant. Therefore, the comprehensive estimate of K_f, calculated from a ratio of the Edemagenic Gain and Edemagenic Time Constant, was minimally affected. The error (~10%) does not appreciably increase if R_L is increased dramatically

(i.e., 100 fold) to simulate the occlusion of lymphatic vessels with organ excision. Similarly, changes in interstitial compliance did not affect the comprehensive K_f estimate. A 50% increase in interstitial compliance led to a 50% increase in the Edemagenic Gain as well as in the Edemagenic Time Constant. This insensitivity of K_f estimation to the change in the particular values of R_L and C is a general property of the comprehensive technique, and can alternatively be shown from *Eqs. 7-9*. In the case of the transient gravimetric technique, nominal values of R_L and C results in 5% error. An incidental 50% increase in C results in 3% error. An incidental 50% increase in R_L results in 3% error. In the case of the steady-state gravimetric technique, nominal values of R_L and C results in 91% error. An incidental 50% increase in R_L results in 89% error. An incidental 50% increase in C results in 91% error.

Effect of change in σ and PS on K_f estimation. Figure 4 illustrates the effect of a simulated incidental decrease in the microvascular reflection coefficient and the protein permeability-surface area product on K_f estimates in sheep lung. In the case of the comprehensive gravimetric technique, a 50% decrease in σ resulted in -3% error in the K_f estimate. Similarly, a 50% decrease in PS resulted in -1% error in the K_f estimate. These errors in K_f estimates were specific to the particular parameter values for sheep lung (Table 1). In the case of the transient gravimetric technique, nominal values of σ and PS result in 5% error. An incidental 50% decrease in σ results in 3% error. An incidental 50% decrease in PS results in 6% error. In the case of the steady-state gravimetric technique, nominal values of σ and PS result in 91% error. An incidental 50% decrease in σ results in 90% error. An incidental 50% decrease in PS results in 89% error.

Effect of errors in measurement of P_c, Π_c, and Π_i on K_f estimation. Surprisingly, the assumption that Π_i was constant only resulted in 5% error in estimated K_f using the comprehensive technique. When it was assumed that Π_i can be measured directly in the course of an experiment, the resulting error in K_f estimation fell to only 3%. Measurement errors have a relatively greater impact. Errors in P_c, Π_c, and Π_i measurements manifest as error in microvascular driving pressure, P_c-$\sigma(\Pi_c - \Pi_i)$. When the estimated composite microvascular driving pressure was given an error of 20%, the steady-state gravimetric technique resulted in 91% error in K_f, and the transient and comprehensive techniques each resulted in 21% error.

Experimental evaluation

Illustration of the novel comprehensive technique using experimental data from the rat. Figure 5 illustrates the novel K_f estimation technique using data collected from the rat small intestine. In this representative experimental data set, a 17.42 cmH_2O increase in microvascular pressure caused a 1.605g increase in the intestinal weight (Fig. 5A). To compensate for weight change due to vascular engorgement with blood (11, 22, 31), the initial rapid change in organ weight (phase with higher slope, Fig. 5B) and the latter slow change in organ weight (phase with lower slope, Fig. 5B) were identified. The Edemagenic Gain was 0.085 ml/mmHg·10g. The Edemagenic Time Constant was 12.42 min. For this illustrative case, K_f estimated using the comprehensive technique (*Eq. 11*) was 0.0065 ml/min·mmHg·10g, whereas K_f estimated using the transient technique (*Eq. 10*) and steady-state technique (*Eq. 9*) were 0.0068 and 0.0016 ml/min·mmHg·10g, respectively. Consistent with previous studies (8), an increase in the small mesenteric

venous pressure was observed within a few seconds (<5 sec) after the manipulation of the snare around superior mesenteric vein. The resulting 15.38±0.44 cmH_2O change in microvascular pressure did not result in accumulation of luminal fluid in any of the six experiments. The resulting values of K_f estimated by the steady-state (*Eq. 9*), transient (*Eq. 10*) and comprehensive techniques (*Eq. 11*) were 0.0015±0.0002, 0.0052±0.0005 and 0.0058±0.0006 ml/min·mmHg·10g, respectively.

Estimation of microvascular filtration coefficient from previously reported data. From the intact dog lung data previously reported by Drake et al. (15), the weight gain during the slow phase was identified and used to estimate K_f using comprehensive and transient techniques. The Edemagenic Gain was 1.72 ml/mmHg·100g, and the Edemagenic Time Constant was 15.3 min. The resulting values of K_f estimated by the steady-state (*Eq. 9*), transient (*Eq. 10*) and comprehensive (*Eq. 11*) techniques were 0.052, 0.11 and 0.11 ml/min·mmHg·100g, respectively.

DISCUSSION

Transient gravimetric technique is valid under specific experimental circumstances. The primary insights of the present work are that the steady-state gravimetric technique has fundamental flaws, and the transient gravimetric technique is sensitive to the duration of the experiment. Selection of an optimal experimental time for the transient gravimetric technique is critical—it cannot be too long or too short. If the experimental time is too short, the measured change in interstitial fluid volume may be confounded by vascular engorgement with blood (31). If the experimental time is too long, the higher order terms in *Eq. 15* cannot be neglected, and the resulting value

becomes sensitive to interstitial compliance and effective lymphatic resistance (Fig. 2). The experimental time for the transient gravimetric approach is reported to be approximately 20 min, which by our analysis is generally long enough to avoid the effects of vascular engorgement but short enough to avoid approximation errors (*Eq. 15*). The Edemagenic Time Constant of an organ is of particular importance since it plays a critical role in the deciding whether the transient gravimetric approach is appropriate for a particular organ. The Edemagenic Time Constant characterizes the interaction of structural properties (K_f, C, and R_L), and determines how fast the interstitial fluid volume reaches steady state. If the Edemagenic Time Constant is too short (as a result of low C or high K_f), the organ weight reaches steady state in a very short period of time, suggesting a very short experimental time period. Therefore, estimating K_f in such organ using the transient technique would incur significant error due to the effects of vascular engorgement. Furthermore, when the lymphatic vessels are tied to eliminate lymphatic return, the Edemagenic Time Constant (*Eq. 8*) is proportional to the interstitial compliance. Therefore, use of the transient gravimetric technique in organs with very low interstitial compliance incurs significant error in estimated K_f (*Eq. 15a*). Tying off the lymphatic vessels not only is experimentally challenging, but can increase estimation error. However, a major finding of the present work is that lymphatic vessels do not have to be tied off for the transient gravimetric approach to be valid. This "modified transient gravimetric technique" can thus avoid tying off lymphatic vessels, which can become problematic for organs or tissues with multiple outlets.

Steady-state gravimetric technique is fundamentally flawed. Because it has been argued that the steady-state gravimetric technique to estimate K_f is confounded by weight

gain from vascular engorgement, formation of large amounts of edema, and the assumption of constant interstitial fluid pressure, it has not been used extensively (14). More importantly, however, the present work has revealed that the steady-state K_f estimation technique represents a myopic approach to interstitial fluid balance. Not only are the profound effects of interstitial fluid storage and lymphatic return neglected, any edema resulting from pharmacological intervention is often attributed to changes in microvascular filtration alone (10). In fact, the present work has revealed for the first time that the conventional steady-state estimation technique yields estimates of K_f that are a function of interstitial compliance and effective lymphatic resistance (*Eq. 14*). Furthermore, when effective lymphatic resistance is significantly elevated, the conventional steady-state K_f estimation technique yields a value that is completely unrelated to K_f, and is instead proportional to interstitial compliance (10). In fact, the steady-state technique may one day gain favor as a novel method to estimate interstitial compliance (10).

Novel technique for estimating K_f. Not only have we evaluated (and suggested improvements to) conventional gravimetric techniques, we have developed a novel technique to estimate K_f. Estimated as the ratio of the Edemagenic Gain and the Edemagenic Time Constant (*Eq. 11*), this simple, comprehensive, and robust technique to estimate K_f from gravimetric data expands the value of the conventional transient technique. First, since the experimental time is determined by the time required to reach steady state, the novel technique does not incur error as a result of selection of too long or too short of an experimental time period (Fig. 2). This could be particularly useful for organs with a very low or high interstitial compliance, since the error is proportional to

interstitial compliance divided by experimental time (*Eq. 15a*). Second, this novel technique provides a second estimate that can be used to corroborate an estimate derived from the transient technique. Although there is no gold standard for gravimetric K_f estimation methods, the close correspondence of the conventional transient estimate and our novel comprehensive estimate may increase the confidence in the resulting value.

Separating organ weight gain due to vascular distention and engorgement. The proposed comprehensive method for estimating K_f, as well as conventional transient method relies upon accurately separating a filtration component of weight gain from vascular volume shifts. When microvascular pressure is increased by raising venous pressure, increase in fluid filtration as well as vascular blood volume lead to higher organ weight. Therefore, if the change in interstitial fluid volume is estimated too soon after a pressure increase, then the change in interstitial fluid volume will be overestimated, leading to an overestimation of K_f. When the organ weight gain due to vascular distention and engorgement is brief, the initial rapid increase in organ weight can be identified using the rate of change of organ weight, consistent with the conventional transient approach (11, 22, 31). However, the critical period of vascular distention can be prolonged (>3 min) by vascular stress-relaxation, which allows vessels to slowly stretch during the experiment (37). The confounding effects of stress-relaxation are particularly problematic in excised organs, and it is possible that excised organs may never reach steady state after microvascular pressure elevation. Therefore, we would not recommend the conventional transient technique or the novel comprehensive technique be used to estimate K_f in excised organs (37). It is of course impossible to prove that any weight gain is only due to changes in interstitial fluid volume and not stress-relaxation without using weight-

independent techniques. A recent report illustrates from comparison of gravimetric and densitometric K_f estimation techniques that larger increases in microvascular pressure (>20 cmH_2O) lead to longer periods of stress-relaxation. However, when the increase in microvascular pressure was <20 cmH_2O, the estimates of K_f using the transient gravimetric technique and laser densitometry converged within 7-9 minutes (37), suggesting that stress-relaxation effects become negligible. Furthermore, the majority of the blood volume shift after elevating mesenteric venous pressure has been reported to occur within 30 sec using weight-independent technique (44). Wallentin found that after the period of vascular stress relaxation (<1 min), the radioactivity levels of tracers used to quantify blood volume were constant suggesting no increase in intestinal blood volume (44). To avoid the effects of stress-relaxation, we thus raised microvascular pressure by 15 cmH_2O, and only used data collected during the slow phase of weight gain in intact rat small intestine.

Kf is not permeability of a vessel. The microvascular filtration coefficient is conventionally viewed as a parameter characterizing the gross behavior of the organ, and is affected by properties of all blood vessels (precapillary arterioles, capillaries and postcapillary venules) involved in water and solute exchange. Furthermore, it is related to the gross permeability of various layers of tissue (e.g., endothelium, vessel walls, and glycocalyx). Defined thus as a lumped parameter, K_f values represent an average of permeability properties both along the vessels (from precapillary arterioles to postcapillary venules) and across various layers of tissue. Although some investigators, motivated by studies of single vessels (32), have attempted to equate K_f to specific mechanical and chemical properties of vessel walls (1, 7, 20, 27), others have treated K_f

more as a "lumped parameter" (11, 14, 15, 22, 28, 39) that relates microvascular driving pressures to microvascular flux. Although it has been relatively common to reduce K_f, characterizing a "black box", into specific physico-chemical parameters such as "hydraulic conductivity" and "surface area", neither is well-defined in a system with heterogeneous hydraulic conductivities, vessel surface areas, and, most importantly, a significant pressure gradient from precapillary arterioles to postcapillary venules. This process of analyzing K_f into physically realizable properties can be continued until every component has been accounted for. The interpretation is of course limited since only a single microvascular driving pressure of an entire organ is assumed. Care must be taken, therefore, not to over-interpret a single value of K_f derived from measurements made from gravimetric approaches applied to an entire organ. K_f, however, retains significant usefulness as a lumped, descriptive parameter, since it can be used to predict how interstitial fluid volume can increase with a known edemagenic stimulus (10), and changes in K_f are related, although indirectly, to physico-chemical properties of the microvasculature.

Assumptions implicit in gravimetric techniques. Because the increase in microvascular pressure is used as an intervention by all gravimetric techniques, the first set of assumptions required by the comprehensive estimation technique is common to all. First, it is assumed that microvascular pressure can be increased rapidly in a stepwise fashion. If microvascular pressure is ramped up too slowly, K_f will be underestimated. Third, it is assumed that P_p, R_L, K_f, and C do not change with increasing microvascular pressure. However, it is possible that these parameters gradually change in response to the elevated microvascular pressure. Limiting the increase in microvascular pressure (~15

cmH_2O) minimizes the change in these parameters. Fourth, it was assumed that Π_c, Π_i and P_{out} do not change with increasing microvascular pressure. Although Π_c would not change with elevated P_c, Π_i decreases as a result of interstitial protein washdown (40), leading to overestimation of K_f. However, recent studies reported that the decrease in Π_i with washdown is lower than previously assumed (40). Thus, when the increase in P_c is small (~15 cmH_2O), the change in Π_i may not be significant. We found that when P_c was elevated by 15 cmH_2O in our detailed numerical simulation, Π_i decreased by less than 2 cmH_2O, resulting in less than 6.36% error in K_f estimation. Also, if P_c is increased by experimentally raising systemic venous pressure (14), the inadvertent increase in lymphatic outlet pressure (P_{out}) could lead to overestimation of K_f. However, if only the organ venous pressure is elevated to increase P_c, the changes in P_{out} and resulting errors in K_f estimation are avoided. These experimental challenges are well-established limitations of conventional K_f techniques, and do not have a particularly negative impact on the novel comprehensive technique (*Eq. 11*).

Necessary assumptions common to all modeling approaches. A second set of assumptions required by the novel comprehensive estimation technique is common to interstitial fluid balance modeling techniques in general. It was assumed that the system is time-invariant—all parameters except the input and output variables (i.e., microvascular pressure and interstitial fluid volume) have constant values. However, it is possible that these parameters slowly change in response to particular pharmacological interventions (10). The errors can be minimized by allowing sufficient equilibration time before estimating K_f (e.g., Figs. 3 and 4). Linearization incurs minimal error if either of the variables varies within a small range, or the relationships are treated as piecewise

linear (5, 8, 31). The interstitial fluid volume-pressure relationship is particularly nonlinear, since the slope increases dramatically at higher hydration levels (28). This particular nonlinearity has minimal impact on K_f estimation (*Eq. 11*), since the contribution of interstitial compliance to transient and steady-state data cancels out when the Edemagenic Gain is divided by the Edemagenic Time Constant. Although these modeling assumptions were necessary to arrive at our novel K_f estimation technique, they are embedded in the three fundamental equations (*Eqs. 1-3*) commonly used to characterize interstitial fluid balance.

Theoretical and experimental evaluation. From our simulated experiments, we conclude that: 1) the comprehensive and transient gravimetric techniques estimated K_f with minimal error in all organs, whereas the steady-state technique resulted in considerable error, 2) the comprehensive and transient gravimetric techniques are unaffected by changes in either interstitial (C), lymphatic (R_L) or microvascular parameters (σ, PS) parameters, and 3) errors in composite microvascular driving pressure lead to proportional errors when using the comprehensive and transient gravimetric techniques, whereas these errors are significantly amplified by the steady-state technique. Evaluation of K_f estimation techniques using experimental data (rat small intestine and dog lung) further corroborates the findings of the simulation results. The value of K_f estimated using the transient technique matches the value of K_f estimated using the comprehensive technique. The steady-state technique results in significantly lower values of K_f compared to comprehensive and transient techniques. The value of K_f we estimated from the rat small intestine was lower than that previously reported (0.114 ml/min·mmHg·100g) by Anzueto et al. (2). Although Anzueto et al. estimated K_f for rat

small intestine using a transient technique, they analyzed their data between 3 and 10 minutes after microvascular pressure elevation. This timeframe can include the effects of vascular blood volume shifts, leading to overestimation of K_f (37). The K_f estimated in dog lung using comprehensive and transient techniques is higher than the value reported (0.07 ml/min·mmHg·100g) by Drake et al (39). The large increases in microvascular pressure required for the K_f estimation technique used in Drake et al.'s study may have led to lower estimates of K_f (39). Limiting the magnitude of the microvascular pressure increase is critical, especially, for isolated organs (34).

The conventional transient and novel comprehensive K_f estimation techniques are not confounded by changes in interstitial fluid storage or lymphatic function. Because interstitial compliance as well as effective lymphatic resistance contributes equally to the Edemagenic Gain and the Edemagenic Time Constant (Fig. 1), they cancel out when using their ratio to estimate K_f (*Eq. 11*). Thus, the estimate of K_f is not only insensitive to the particular values of interstitial compliance and effective lymphatic resistance, but to any incidental changes in their values that occurs before pressure elevation. This is particularly important, because it has been common practice to sever or ligate lymphatic vessels during organ excision (14). Similarly, the conventional transient gravimetric technique becomes insensitive to changes in interstitial compliance and lymphatic return when the experimental time period is short (*Eq. 15,* Fig. 2). The conventional transient and novel comprehensive techniques not only allow more accurate estimation of K_f, but also provide a means to detect changes in K_f that may otherwise be masked by compensatory changes in interstitial and lymphatic function.

ACKNOWLEDGEMENTS

Portions of this work were supported by National Institutes of Health Grants K25HL070608 (C. M. Quick), AHA-0565116Y (C. M. Quick), AHA-0365127Y (R. H. Stewart), and CDC-623086 (G. A. Laine).

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TABLE

Table 1 Comparison of K_f estimates using comprehensive (*Eq. 11*), transient (*Eq. 10*) and steady-state (*Eq. 9*) gravimetric techniques. In each animal model, interstitial fluid volume was simulated in response to a 50% increase in microvascular pressure using a complex, realistic model of interstitial fluid volume regulation described elsewhere (9). Using transient and steady-state change in simulated interstitial fluid volume, K_f was estimated using comprehensive (*Eq. 11*), transient (*Eq. 10*) and steady-state (*Eq. 9*) gravimetric techniques. Percent error in estimated K_f is determined with respect to assumed value of K_f (*Eq. 12*).

FIGURES

Figure 1 Illustration of change in interstitial fluid volume (ΔV) in response to a step increase in microvascular pressure (ΔP_c). The Edemagenic Gain (EG) determines the final change in interstitial fluid volume between two steady states and thus the steady-state behavior of the system. The Edemagenic Time Constant (τ) determines the rate at which interstitial fluid volume changes and thus the transient behavior of the system.

Figure 2 Illustration of the effect of experimental time (Δt) on K_f estimation using the transient gravimetric technique. Δt is the time for which the simulated organ weight gain data is analyzed after microvascular pressure is elevated (*Eq. 10*).

Figure 3 (A) Simulated increase in interstitial fluid volumes with a 50% increase in microvascular pressure used to illustrate possible confounding effects of incidental increases in effective lymphatic resistance (R_L) and interstitial compliance (C) on K_f estimation. Simulation results from numerically evaluating a complex, realistic model reported elsewhere (9) using parameters for the sheep lung (Table 1). (B) Comparison of estimated K_f values using the novel comprehensive technique (*Eq. 11*), the conventional transient gravimetric technique (*Eq. 10*), and the conventional steady-state gravimetric technique (*Eq. 9*). The true value of K_f is 0.014 ml/min·mmHg·100g.

Figure 4 (A) Simulated increase in interstitial fluid volumes with a 50% increase in microvascular pressure used to illustrate possible confounding effects of incidental changes in microvascular permeability to protein on K_f estimation. Simulation results from numerically evaluating a complex, realistic model reported elsewhere (9) using parameters for the sheep lung (Table 1). (B) Comparison of estimated K_f values using the novel comprehensive technique (*Eq. 11*), the conventional transient gravimetric technique (*Eq. 10*), and the conventional steady-state gravimetric technique (*Eq. 9*). The true value of K_f is 0.014 ml/min·mmHg·100g.

Figure 5 Illustration of K_f estimation with comprehensive approach using data in rat small intestine. (A) Increase in small intestine weight in response to a 12.8 mmHg increase in microvascular pressure. (B) Separation of rapid phase (phase with initial sharp slope) from slow phase of weight increase to compensate for vascular distention and engorgement with blood. (C) Edemagenic Time Constant (τ=12.43 min) is estimated using the slope of the regression line after taking natural log of weight gain during slow phase as a function of time. The time to reach steady state was taken to be the time when tissue weight changed by less than 3mg/min/10g, within the resolution of our equipment in our experimental preparation.

Animal Model	Parameter Values	Comprehensive Technique		Steady-State Gravimetric Technique		Transient Gravimetric Technique	
		K_f (ml/min·mmHg/100g)	% error	K_f (ml/min·mmHg/100g)	% error	K_f (ml/min·mmHg/100g)	% error
Sheep Lung	**K_f = 0.014 ml/min·mmHg·100g** (17), R_L = 100 mmHg·min/ml·100g (30), P_c = 14.34 mmHg (17), C_c = 74 mg/ml (17), σ = 0.48 (36), PS = 0.02 ml/min·100g (36), P_p = 20 mmHg, P_{out} = 2 mmHg	0.0133	5	0.0012	91	0.0133	5
Dog Lung	**K_f = 0.07 ml/min·mmHg·100g** (16, 24), R_L = 76 mmHg·min/ml· 100g (12), P_c = 7 mmHg (21), C_c = 58 mg/ml (26), σ = 0.62 (36), PS = 0.07 ml/min·100g (36), P_p = 20 mmHg, P_{out} = 2 mmHg	0.064	9	0.0059	92	0.062	11
Dog Skeletal Muscle	**K_f = 0.007 ml/min·mmHg·100g** (4), R_L = 200 mmHg·min/ml·100g (29), P_c = 24 (19), C_c = 54 mg/ml (45), σ = 0.72 (36), PS = 0.03 ml/min·100g (36), P_p = 25 mmHg, P_{out} = 2 mmHg	0.0066	6	0.00066	91	0.0065	6

Table 1 Comparison of K_f estimates using comprehensive (*Eq. 11*), transient (*Eq. 10*) and steady-state (*Eq. 9*) gravimetric techniques. In each animal model, interstitial fluid volume was simulated in response to a 50% increase in microvascular pressure using a complex, realistic model of interstitial fluid volume regulation described elsewhere (9). Using transient and steady-state change in simulated interstitial fluid volume, K_f was estimated using comprehensive (*Eq. 11*), transient (*Eq. 10*) and steady-state (*Eq. 9*) gravimetric techniques. Percent error in estimated K_f is determined with respect to assumed value of K_f (*Eq. 12*).

Figure 1

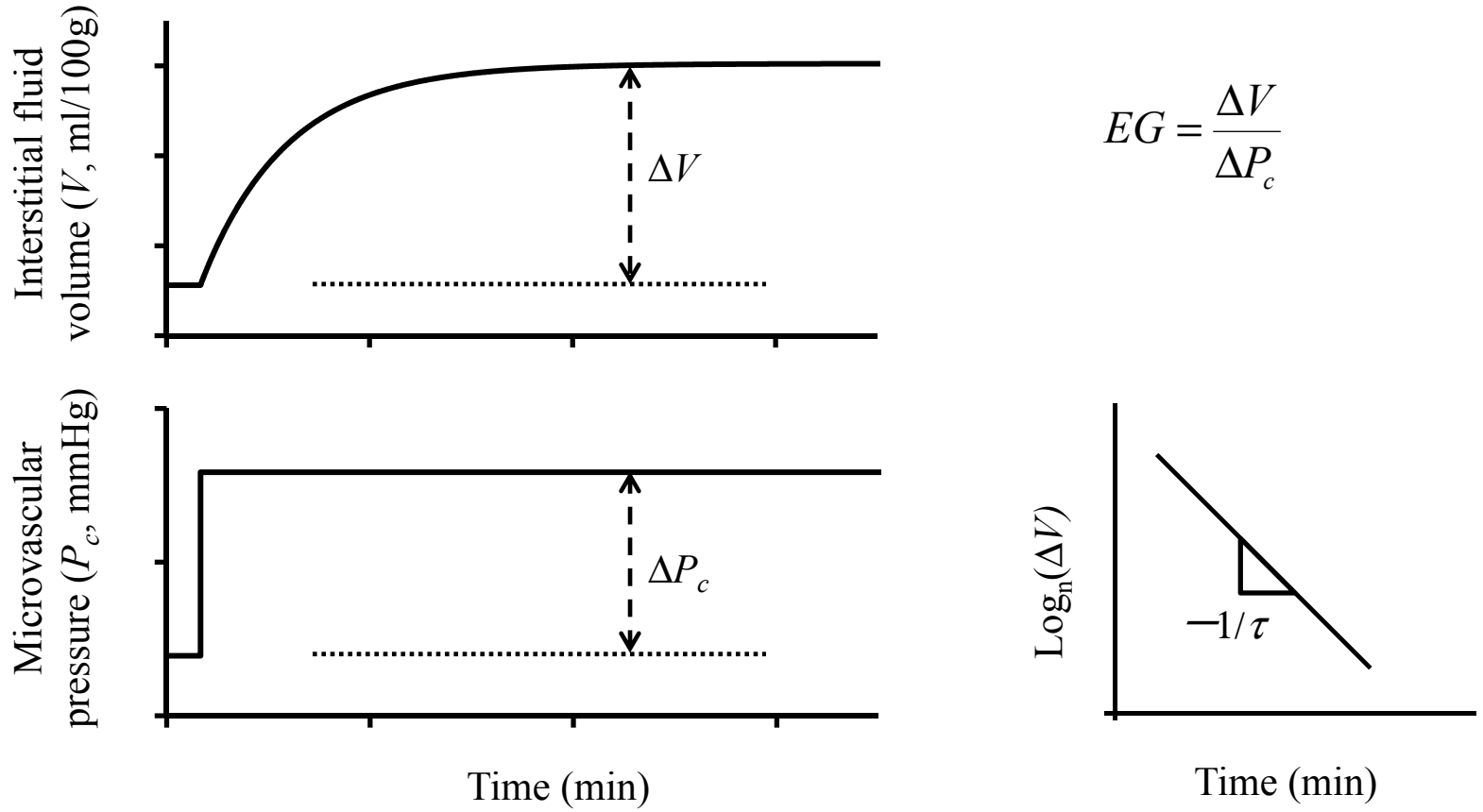
Interstitial fluid volume (V, ml/100g)
ΔV
Microvascular pressure (P_c, mmHg)
ΔP_c
Time (min)
$EG = \frac{\Delta V}{\Delta P_c}$
$\mathrm{Log_n}(\Delta V)$
$-1/\tau$
Time (min)

Figure 2

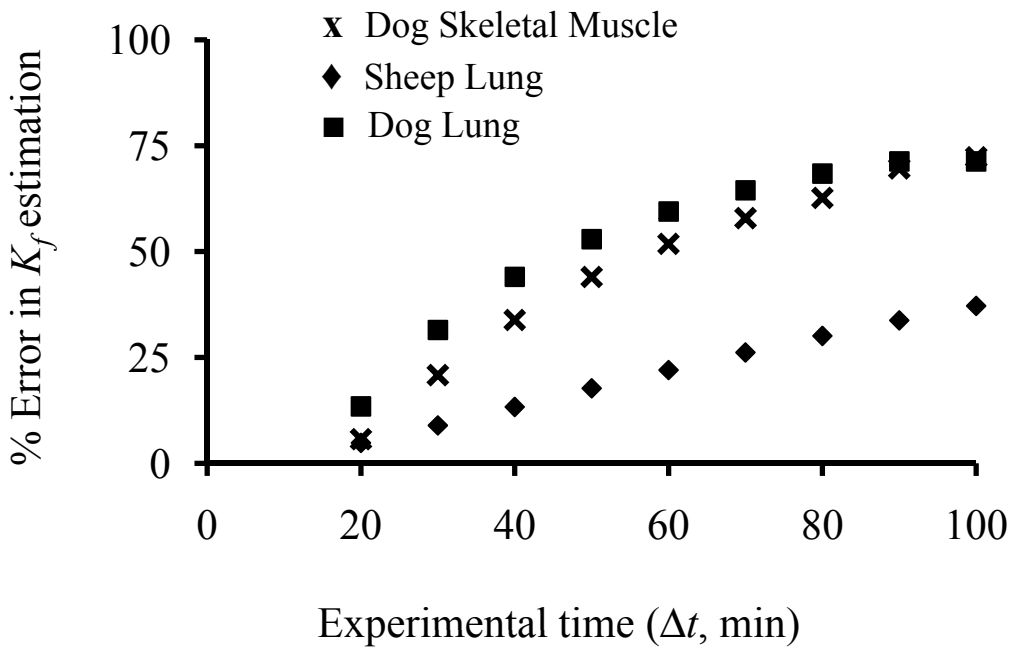
x Dog Skeletal Muscle
♦ Sheep Lung
■ Dog Lung
% Error in K_f estimation
100
75
50
25
0
0
20
40
60
80
100
Experimental time (Δt, min)

Figure 3A

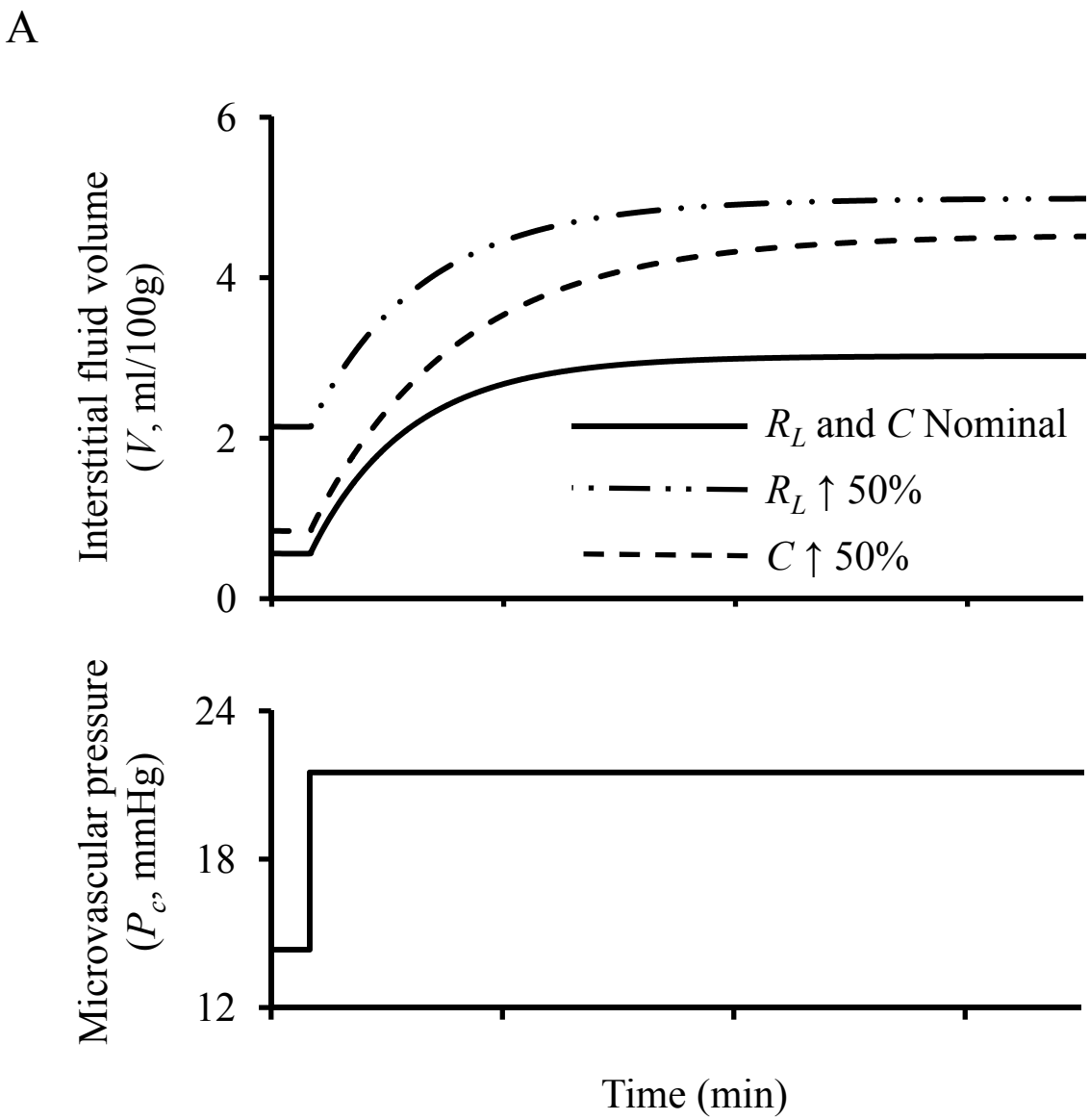
A
6
4
2
0
Interstitial fluid volume
(V, ml/100g)
R_L and C Nominal
R_L ↑ 50%
C ↑ 50%
24
18
12
Microvascular pressure
(P_c, mmHg)
Time (min)

Figure 3B

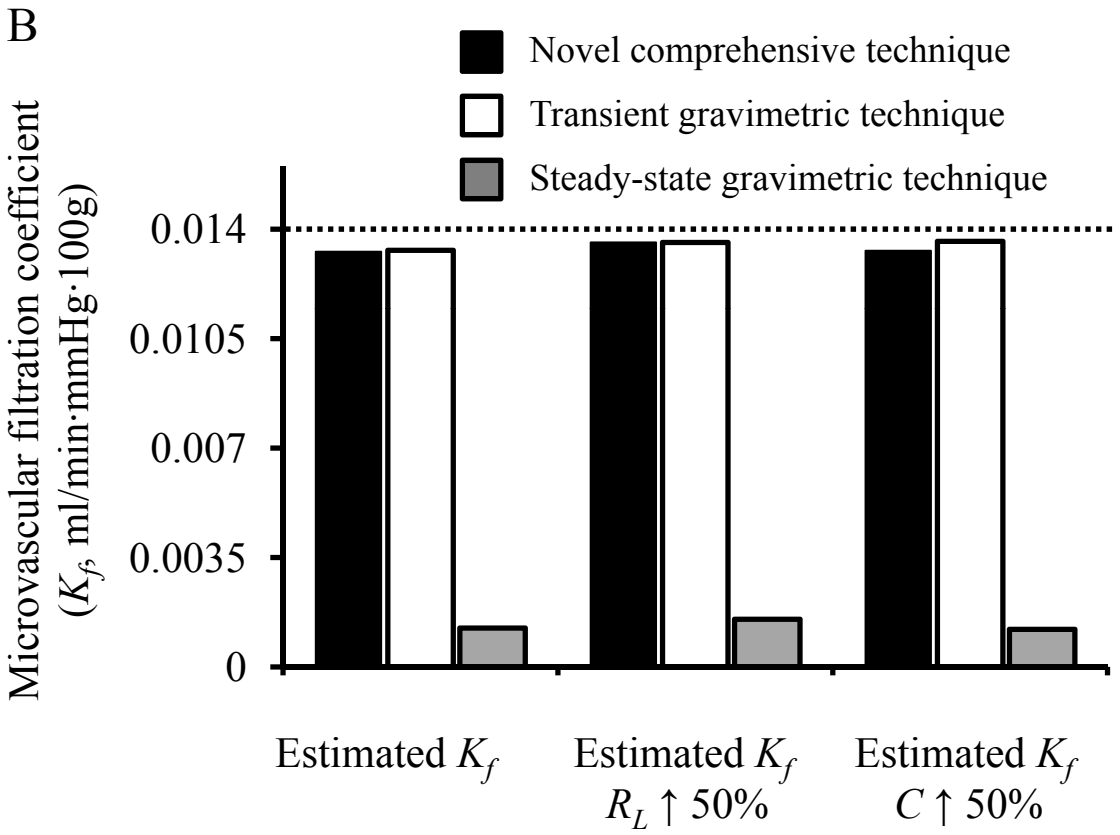
B
Novel comprehensive technique
Transient gravimetric technique
Steady-state gravimetric technique
Microvascular filtration coefficient
(K_f, ml/min·mmHg·100g)
0.014
0.0105
0.007
0.0035
0
Estimated K_f
Estimated K_f
R_L ↑ 50%
Estimated K_f
C ↑ 50%

Figure 4A

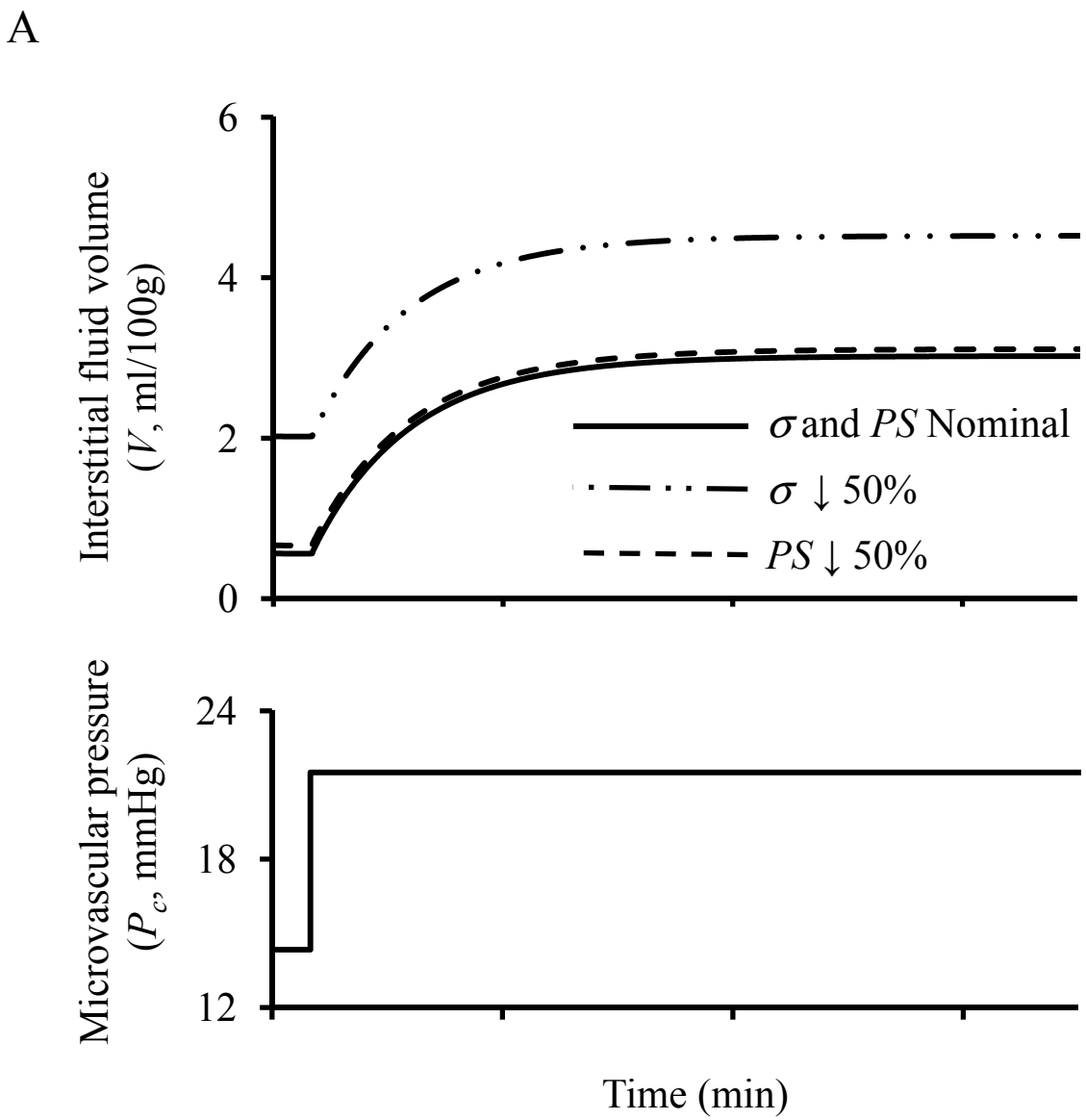
A
Interstitial fluid volume
(V, ml/100g)
6
4
2
0
σ and PS Nominal
$\sigma \downarrow 50\%$
$PS \downarrow 50\%$
Microvascular pressure
(P_c, mmHg)
24
18
12
Time (min)

Figure 4B

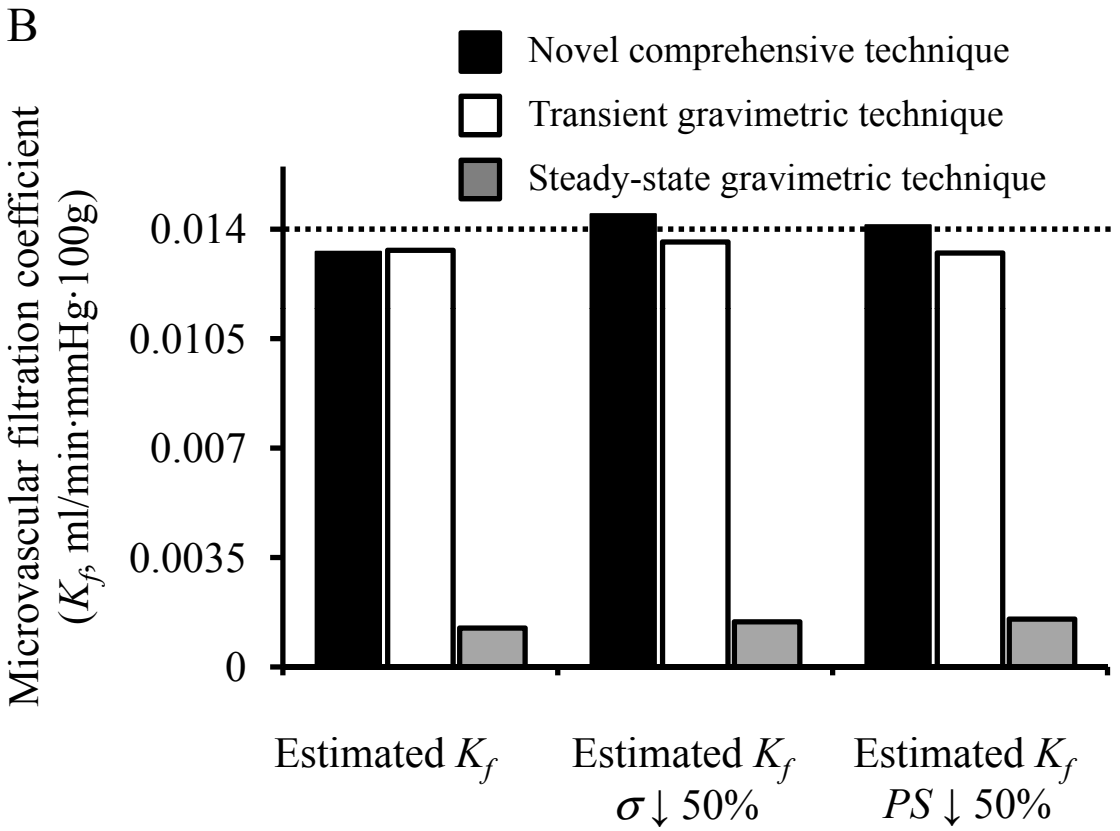
B
Novel comprehensive technique
Transient gravimetric technique
Steady-state gravimetric technique
Microvascular filtration coefficient
(K_f, ml/min·mmHg·100g)
0.014
0.0105
0.007
0.0035
0
Estimated K_f
Estimated K_f
$\sigma \downarrow$ 50%
Estimated K_f
$PS \downarrow$ 50%

Figure 5A

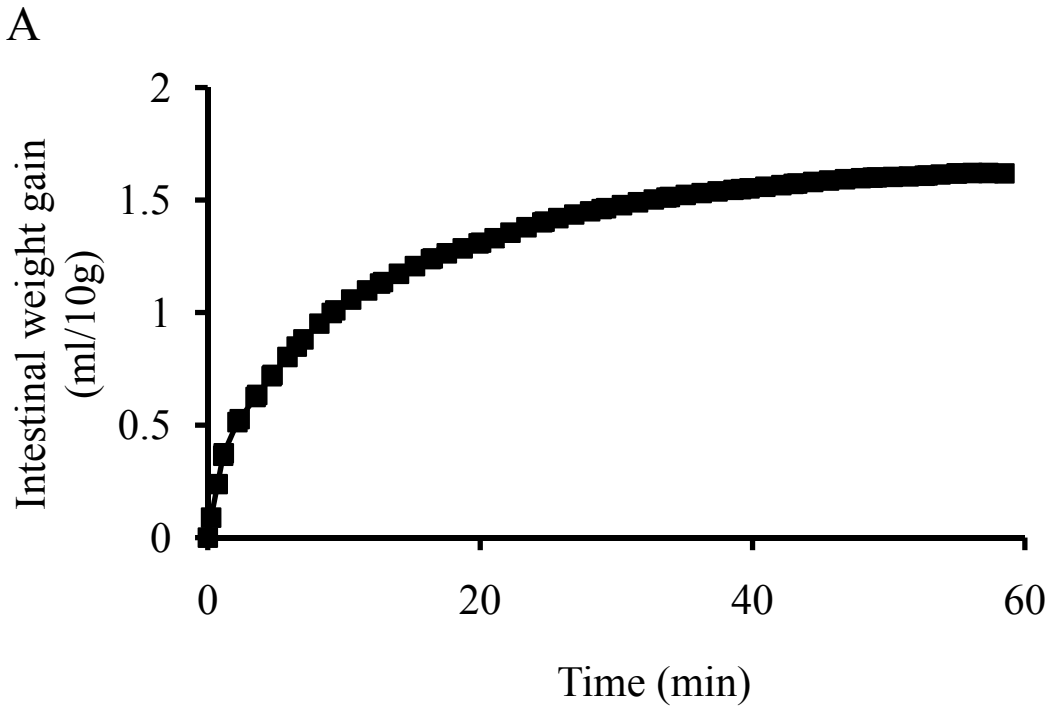

Figure 5B

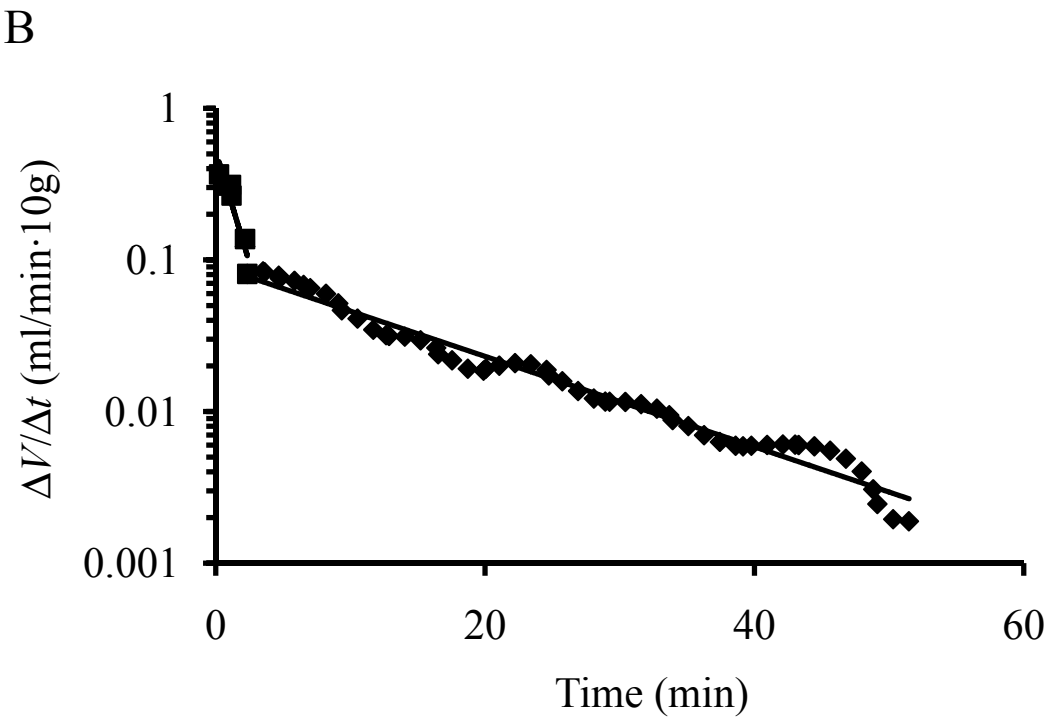

Figure 5C