Virus Adsorption Within Pores in Latex: Assessment of Reversibility Effects

Matthew R. Myers, C. David Lytle, and Licia B. Routson
Center for Devices and Radiological Health, U. S. FDA, Rockville, MD 20852

Recently, a mathematical model for simulating virus transport through synthetic barriers (gloves, condoms, instrument sheaths, etc.) was developed (Myers et al., 1999, hereafter referred to as MLR99). The mathematical model comprises a convective-diffusion equation modeling the dominant transport mechanisms away from the barrier surface, and a ''reaction-rate'' boundary condition containing information (empirically determined for each virus/suspending-fluid/barrier-material combination of interest) characterizing the short-range colloidal force between the virus and the barrier. The reaction-rate boundary condition is derived from the perfect-sink model of adsorption, in which viruses contacting the pore wall are assumed to be irreversibly adsorbed. In comparison with data from experiments using bacterial viruses and latex membranes in a parallel-plate channel, the model considerably underpredicted the rate of virus transmission for residence times that were large compared to the time necessary for the virus to diffuse the height of the channel. Data from transmission experiments reported in MLR99 are reproduced by the square symbols in Figure 1. (Data to be discussed subsequently are also shown.) The horizontal axis represents the ratio of the average residence time to diffusion time for the bacterial virus PRD1 (65-nm diameter), where the residence time is the volume of the channel divided by the measured flow rate of suspending fluid, and the diffusion time is the square of the channel half-height divided by the Brownian diffusivity for a spherical particle in an unbounded medium. The vertical axis represents the number of viruses exiting the channel in a short time period divided by the number of viruses entering the channel in the same period. The solid line shows the model predictions obtained through a finite-difference solution to the convective-diffusion equation and reaction-rate boundary condition in the parallel-plate geometry. The higher rate of transmission observed experimentally (at larger relative times) could reasonably be attributed to reversibility—desorption of viruses back into suspension—or to saturation of potential virus adsorption sites. A desire to determine the extent to which reversibility and saturation limit the applicability of the perfect-sink model of virus adsorption to latex motivated a new set of virus-transport experiments.

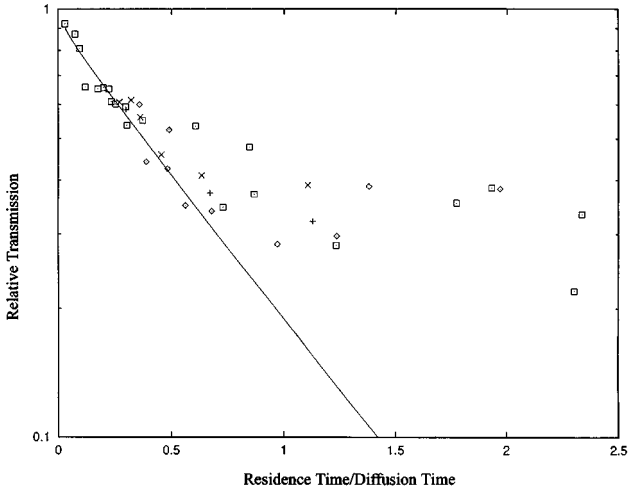

Figure 1. Relative transmission of PRD1 virus through the parallel-plate channel as a function of relative time, for input concentrations of 10^3/mL (◇), 7×10^3/mL (+), 10^5/mL (□), and 10^7/mL (×).

The theoertical prediction based on the perfect-sink approximation is given by the solid line.

Experiments

In the experiments, the basic procedure described in MLR99 for model calibration was followed. Latex material was wrapped around two plastic rectangular plates, and a channel was formed by clamping the two plates together with spacers in between. The channel height (distance between the latex films) was approximately 100 μm, and the width and length of the channel were about 100 and 1,000 times this value, respectively. The suspending fluid was physiologic (0.16

Correspondence concerning this article should be addressed to M. R. Myers.

M) saline in each case. PRD1 virus was used throughout, and the suspension flowed through the vertically positioned channel under gravity. Drops exiting the channel were assayed for their virus content.

Reversibility or saturation occurring within the experiments would be manifested in an increase in the relative transmission as a function of time, with an eventual 100% transmission rate resulting when equilibrium is established between adsorption and desorption. The relative transmission was thus recorded as a function of time, by assaying select drops exiting the channel throughout the experiment. Five of the ''time-dependence'' experiments were performed for durations of 16, 31, 46, 52, and 61 min. These durations were chosen to exceed the duration of the transmission experiments of MLR99, which was typically between 15 and 30 min. The mean residence time/diffusion time was 0.74, and the virus concentration was 10^5/mL [a high titer of HIV in plasma (Clark et al., 1991)]. To further study the nature of the binding between viruses and latex, the experiments were followed by attempts to elute the bound viruses via two different rinsing procedures. Following each of the five transmission phases, the channel was rinsed first with pure suspending fluid (saline), and the virus content of the rinsate measured. Subsequent to this rinse, a second rinse was performed with a solution of saline mixed with the nonionic surfactant. Tween 80 (Sigma Inc.) at a concentration of 0.1%, and the viruses freed by this second rinse were counted.

The time (both the absolute time after initiation of the experiment and the relative time) at which saturation becomes observable should be dependent upon virus concentration input into the apparatus, with deviations from the perfect-sink model occurring at earlier times for higher concentrations. To test for this nonlinear effect of saturation, ''concentration-dependence'' experiments were performed at virus titers of 10^3/mL, 7×10^3/mL, and 10^7/mL. The relative transmissions were measured at relative times ranging between 0 and 2.

Results

For the time-dependence experiments, the relative transmission was found to be constant for each experiment, after a transient period of approximately 5 min. (Time traces available from authors.) The number of viruses missing—entering the channel but not counted in the transmitted volume of suspending fluid—is plotted in Figure 2. Following these experiments, essentially none of the bound virus was recovered by the saline rinse. For the rinse with the surfactant Tween 80, the number of viruses recovered is plotted in Figure 2, as a function of experiment duration. The numbers of recovered viruses and missing viruses are approximately equal (average difference = 15%). The dependence of the number of eluted viruses as a function of time (experiment duration) is roughly linear, as evidenced by the line of best fit through the five recovered-virus data points.

The relative transmission as a function of relative time for the three input concentrations considered, as well as for the original concentration of 10^5/mL, is plotted in Figure 1. To within the inherent variations occurring in virus-transport experiments, there appears to be no difference between the various concentrations in either the relative transmissions or the relative time at which the measured transmissions begin to deviate from the theoretical line derived using the perfect-sink approximation in the parallel-plate channel.

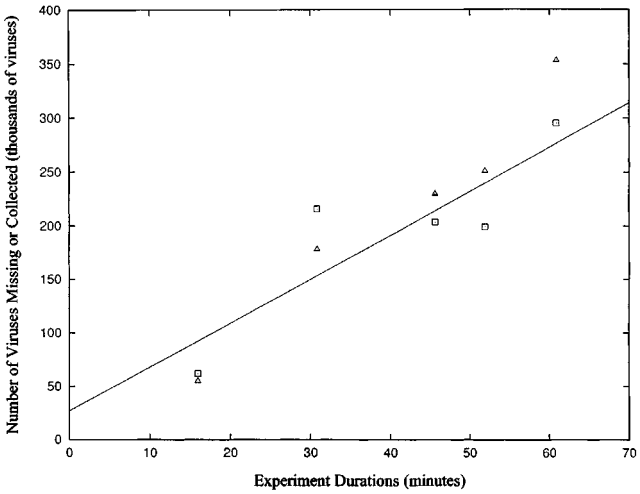

Figure 2. Number of viruses missing (entering the channel but not counted in the exiting volume) during the transmission experiments of duration 16, 31, 46, 52 and 61 min (triangular symbols).

Also plotted are the number of viruses recovered (square symbols) via a rinse with the surfactant Tween 80 during the same experiments. Solid line is the best-fit line through the recovered-virus values.

Discussion

The absence of a gradual increase in transmission rate with time (asymptotically approaching 100%), combined with an independence of transmission with inlet concentration over a 10,000-fold range, indicates that reversibility and saturation are not significant in the transmission experiments. (The independence of the transmission upon virus concentration is also evidence that the suspension is sufficiently dilute to ignore virus/virus interactions). Rather, the linear dependence on time of the accumulated amount of bound virus (Figure 2), that is, a constant adsorption rate, is characteristic of the irreversible adsorption into a perfect sink (van de Ven, 1989). The binding of PRD1 virus to latex appears to be due in large part to nonionic forces (such as van der Waals), which are reduced by the nonionic surfactant.

In the preceding experiments, the maximum surface concentration of PRD1 viruses bound to the latex (occurring with the 10^7/mL inlet concentration) was approximately 10^7/cm^2. For PRD1 adsorption to latex in a medium of physiologic saline, the perfect-sink model can be expected to hold at least up to this surface concentration. In the example considered in MLR99 of a 1-μm diameter cylindrical pore in a latex barrier, under the influence of a steady 80,000 dyn/cm^2 test pressure, the concentration of viruses on the pore wall after a 30-min duration is roughly 10^6/cm^2, within the range of validity of the perfect-sink assumption.

An alternative hypothesis for the divergence between experiment and theory in Figure 1 is a high sensitivity of virus

transmission rate to channel geometry. Suppose, for example, that the separation between the latex sheets is not uniform across the channel. A nonuniform separation might result if a large clamping compression caused the sheets to be closer in the middle of the channel than at the transverse boundaries, where the spacer thickness determines the local channel height. At locations where the channel height is largest, the virus flux can be substantially larger than at other locations, because more fluid flows through the larger-height section, and more importantly the time necessary for viruses to migrate to the channel wall and adsorb is higher. The enhanced flux through the larger-height sections could result in a higher overall virus transmission than a uniform channel producing the same flow of suspending fluid. The sensitivity to channel geometry is expected to be strongest for smaller channel heights, that is, for larger residence time/diffusion time values. This alternative hypothesis has indeed been found to be viable and is explored elsewhere (Myers, 2000).

The conclusions of this article are based upon experiments using the bacterial virus PRD1. However, the relative transmission vs. relative time curves for other bacterial viruses, including $\phi 6$ and MS2 (MLR99), closely resemble that of PRD1 (Figure 1): the transmission data track the predictions from the perfect-sink-based model up to a relative time of about 0.5, at which point a "knee" occurs where the measured transmission values begin to exceed the theoretically predicted ones. This behavior was also observed for the one human virus for which the model has been calibrated, herpes simplex virus.

Since at this stage the model has not been calibrated [the "rate constant" characterizing the virus/barrier interaction force determined (MLR99)] for human viruses, such as HIV or the hepatitis viruses, or for any virus in blood or semen, no definite conclusions can be made for these important conditions. However, given the high adsorptivity of PRD1 [at a relative time of only 1 in the parallel-plate channel, the transmission is less than 20% (Figure 1)] already observed, for these other conditions it is likely that at least saturation at the latex surface will not invalidate the perfect-sink assumption.

Acknowledgments

We acknowledge financial support from the Office of Women's Health of the U. S. Food and Drug Administration. The authors also thank the reviewer of MLR99, who suggested the reversibility hypothesis studied in this article.

Literature Cited

Clark, S. J., M. S. Saag, W. D. Decker, S. Campbell-Hill, J. L. Roberson, P. J. Veldkamp, J. C. Kappes, B. H. Hahn, and G. M. Shaw, "High Titers of Cytopathic Virus in Plasma of Patients with Symptomatic Primary HIV-1 Infection," *N. Engl. J. Med.*, **324**, 954 (1991).

Myers, M. R., C. D. Lytle, and L. B. Routson, "A Mathematical Model for Simulating Virus Transport Through Synthetic Barriers," *Bull. Math. Biol.*, **61**, 113 (1999).

Myers, M. R., "Calculation of Brownian Particle Transmission in Long, Wide Channels," *AIChE J.*, in press (2000).

Van de Ven, T. G. M., *Colloidal Hydrodynamics*, Academic Press, San Diego (1989).

Manuscript received Sept. 7, 1999, and revision received Mar. 10, 2000.