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# Label-Free Measurements of Tenofovir Diffusion Coefficients in a Microbicide Gel Using Raman Spectroscopy

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ABSTRACT

Confocal Raman spectroscopy was implemented in a new label-free technique to quantify molecular diffusion coefficients within gels. A leading anti-HIV drug, tenofovir, was analyzed in a clinical microbicide gel. The gel was tested undiluted, and in 10%-50% wt/wt dilutions with vaginal fluid simulant to capture the range of conditions likely occurring *in vivo*. The concentration distributions of tenofovir in gel over time and space were measured and input to a mathematical diffusion model to deduce diffusion coefficients. These were $3.16 \pm 0.11 \times 10^{-6}$ $cm^2/s$ in undiluted gel, and increased by 11%-46% depending on the extent of dilution. Results were interpreted with respect to traditional release rate measurements in devices such as Franz cells. This comparison highlighted an advantage of our assay in that it characterizes the diffusive barrier within the gel material itself; in contrast, release rate in the traditional assay is affected by external conditions, such as drug partitioning at the gel/liquid sink interface. This new assay is relevant to diffusion in polymeric hydrogels over pharmacologically relevant length scales, for example, those characteristic of topical drug delivery. Resulting transport parameters are salient measures of drug delivery potential, and serve as inputs to computational models of drug delivery performance.



## Introduction

Microbicides can provide an alternative (to vaccines) and important modality for prevention of sexually transmitted HIV and possibly other pathogens.[1] They occupy a unique space in the HIV prevention network, and are among a small number of prevention methods that could be used independently or in conjunction with other forms of prophylactics. When applied vaginally, they are under primary control of a female user, who would not have to rely on her sexual partner to provide HIV protection. Multiple drugs and delivery vehicles are being evaluated for potential microbicide products. The most advanced are a tenofovir gel and a dapivirine ring, both of which have demonstrated efficacy in at least one trial.[2,3] Both drugs act within the vaginal, and potentially rectal, mucosa by inhibiting reverse transcriptase production by virions in early stages of their interaction with infectable host cells. Results from gel trials have been mixed, in part because of limitations of user adherence.[4] Still, the familiarity of vaginal gels to women and their current use for multiple gynecological purposes motivate continued evaluation of them as a modality for delivery of microbicides.

Initial analysis of the drug delivery capability of prototype products typically includes measuring the release rate of the drug from its vehicle.[5-7] This is often performed in a vertical diffusion cell (e.g., a Franz cell) which exposes an upper gel layer to a lower stirred fluid sink compartment, and measures the accumulating drug concentration in the sink versus time. For standardized testing in which the gel layer thickness and the drug permeability of the supporting membrane in the Franz cell are invariant, this release rate can be used meaningfully to compare prototype products for a given drug. However, the release rate parameter cannot be used to predict drug delivery by a gel into target tissues, only to contrast its potential with that of other gels. Accurate prediction is central to rational product design, and benefits substantially from a deterministic, analytic approach to the drug delivery process. This includes not just release from the vehicle but drug transport into and through target tissue. Computational modeling can help

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achieve this. The modeling requires input parameters including fundamental transport properties, such as drug diffusion and partition coefficients, in all compartments of the problem.[8-10] While modeling predictions can provide comprehensive understanding of how product properties govern drug delivery, the drug diffusion coefficient in a gel per se can be a more robust alternative to the traditional release rate parameter in characterizing drug delivery potential.

In pharmacological processes, drugs may migrate through gels that are not just their vehicles but are also target matrices, for example, the vitreous of the eye or the cervical mucus.[11] Transport often occurs over length scales on the order of a few hundred microns. Drug diffusion in gels is sensitive to the length scale over which it is evaluated.[12] However, there has been relatively little work on measuring diffusion over the intermediate length scale relevant to the microbicide delivery problem. Such knowledge is vital to understanding microbicide gel functionality and to development and evaluation of candidate products. This study was designed to address this shortcoming. Using a custom-designed chamber and optical system, we applied confocal Raman spectroscopy (CRS) to measure the spatiotemporal history of tenofovir transport within a clinical gel.

RS has been shown to be of practical value for concentration profiling in polymeric matrices.[13,14] This technique detects the frequency change of photons that undergo Raman scattering, a form of inelastic scattering that results from changes in the vibrations of chemical bonds.[15,16] The intensity of Raman scattering is proportional to the concentration of the detected molecule or chemical bond in solution.[17] RS has begun to emerge as a useful tool to obtain molecular concentration profiles in biomedical systems including fluids, gels, and tissues. For example, Kwak and Lafleur[13] used RS to study the diffusion of polyethylene glycol of different molecular weights through calcium-alginate gels. Recently, our group applied high-resolution CRS to measure concentrations of microbicide drugs in liquids and their concentration distributions in porcine tissues, using a Transwell configuration.[18,19] Here, we constructed a custom horizontal diffusion cell enabling measurements of spatiotemporal concentration distributions of the microbicide drug tenofovir in its clinical vaginal gel.[2,20,21] These data were fit with a time-dependent diffusion model to obtain diffusion coefficients. The gels were tested undiluted, and at 10% wt/wt (1:9 VFS:gel) to 50% wt/wt (1:1 VFS:gel) dilutions with vaginal fluid simulant (VFS).[22] These dilutions simulated the range of conditions experienced by the gel within the vaginal canal *in vivo*.[23] Gel dilution and possible gel swelling associated with dilution could alter drug diffusion coefficients and, therefore, transport of drug within and out from the gels.

## Materials and Methods

### *Materials and Diffusion Chamber*

Tenofovir gel and a drug-free placebo gel of the same composition were kindly provided by Professor Lisa C. Rohan (University of Pittsburgh School of Pharmacy). The tenofovir gel was developed by the CONRAD Program (Arlington, VA) for use in clinical studies.[2,20,21] The gel (pH 4.4) was formulated with 1% wt/wt tenofovir (molecular weight 287), and 20% wt/wt glycerol, hydroxyethylcellulose, edetate disodium, citric acid, methylparaben, propylparaben, and purified water. Both tenofovir and placebo gels were diluted with VFS (pH 4.2)[22] to investigate the effect of dilution on gel transport properties. Dilution samples were prepared to contain 10%, 20%, 30%, or 50% by weight of VFS and 90%, 80%, 70%, or 50% by weight of gel, respectively. These dilutions simulated the range of physiological dilutions expected *in vivo*.[22-24] Each sample was thoroughly mixed by vortexing. A gel diffusion chamber was constructed specifically for use in these experiments (Fig. 1). It consisted of 2 custom-manufactured quartz rectangular capillary cells (Starna Cells, Atascadero, CA) that were placed in an aluminum mounting. Each cell had an internal rectangular channel running through the center of the cell with dimensions of 0.3 mm × 8 mm × 20.1 mm. One cell acted as the donor compartment while the other was the receptor compartment. The contact surface between the 2 cells served as the interface across which drug diffused from the donor compartment into the receptor compartment.

### *Diffusion Experiments*

Gel was loaded into each diffusion cell with a syringe and silicone tubing. The donor cell was loaded with the tenofovir gel while the receptor compartment was loaded with the placebo gel. Care was taken during gel loading to avoid the formation of bubbles in the medium, which could affect the molecular diffusion of the target drug. The noninterface ends of the cells were securely sealed with a waterproof tape to prevent gel dehydration. The diffusion chamber was placed on a temperature-controlled motorized stage to maintain the gel at physiological temperature, 37.0 ± 0.5°C, throughout each experiment. A custom-built CRS-OCT (CRS and optical coherence tomography) instrument integrated with a white light camera was used to acquire data in this study.[19] The axial and lateral resolutions of the CRS subsystem are 19 and 3 μm, respectively. The OCT subsystem has an axial resolution of 8 μm and a lateral resolution of 40 μm, and the white light camera offers a lateral resolution of 2 μm. Prior to CRS measurements, the OCT subsystem was used to visualize the diffusion chambers to verify proper alignment of their central channels. The white light camera

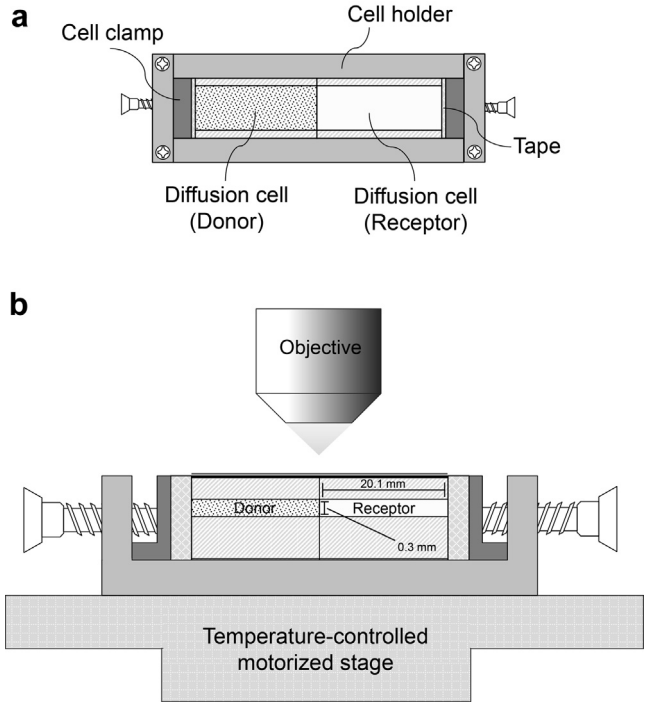


**Figure 1.** Custom-built diffusion chamber used to perform the gel diffusion experiments. (a) Schematic of the diffusion cell for measuring drug transport and the diffusion coefficients in gels. The donor compartment is loaded with tenofovir gel, and the receptor is filled with a placebo gel of the same composition, minus the drug. (b) Side view of the diffusion cell that is set up on a temperature-controlled motorized stage.