Introduction

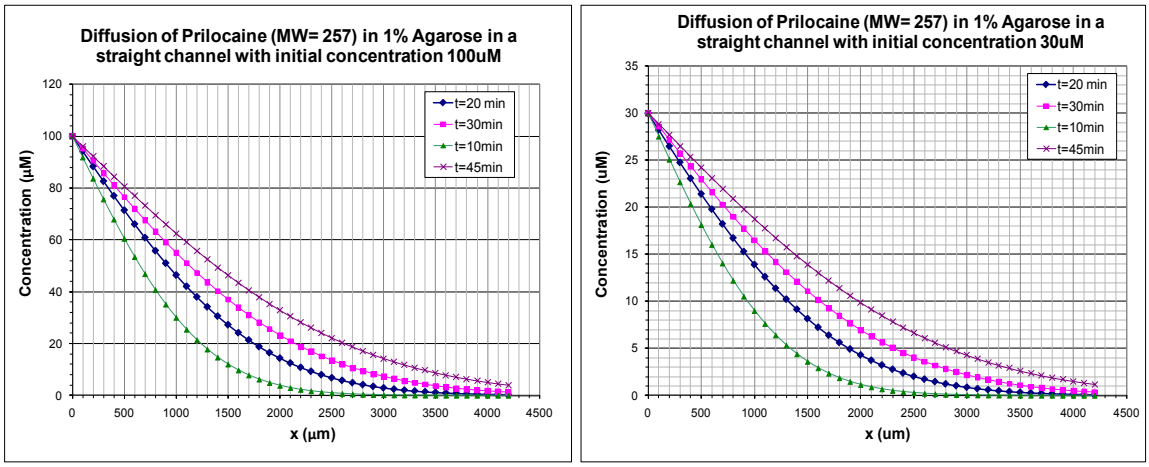
This supplementary file has been created to present a study on the isolation condition of the drug within the drug well. All experiments were completed using the same assay setup described in the manuscript; however, no worms were inserted and the drug was replaced by a concentration of Fluorescein – a fluorescing dye with a pK_3 of 8.0 and molecular weight of 332 g/mol (although slightly larger than levamisole (MW 204.25 g/mol)), this weight is a good representation of smaller anthelmintics). Within the 8.8 pH of the channel medium, we expect ~90% ionization (to 2^-). The dye was excited using white light coupled with a GFP filter at 470 nm to induce fluorescence.

Diffusion

Both theoretical (SF3a) and experimental (SF3b-f) diffusion work was done to illuminate diffusion effects near the chemical interface. We note this work is not representative of the net movement of a charged drug as electro-osmotic and electro-phoretic forces will always be present in this assay. However, this work is important to assess the device's performance for neutral anthilmentics (i.e. Benzimidazoles).

*Note: a majority of anthelmintics have a positive charge (1^+ in >8.0 pH) (i.e. Nicotinics). Only some exotic anthelmintics have negatives charges (e.g. quisqualate and Kainite); however, these drugs are not used and need not be taken into account.

$$c(\mathrm{x},\mathrm{t}) = c_0 * erfc\left(\frac{x}{2\sqrt{Dt}}\right)$$

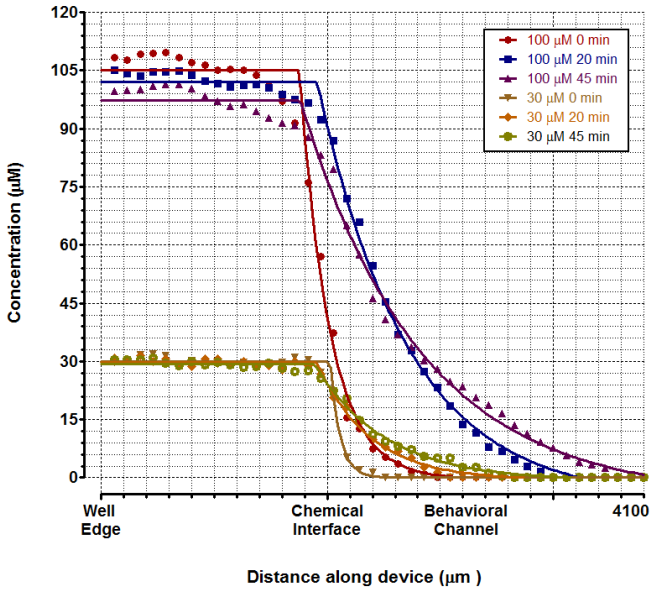


SF3a Theoretical diffusion calculations for priloclane across a straight channel filled with 1% w/v agarose/water.

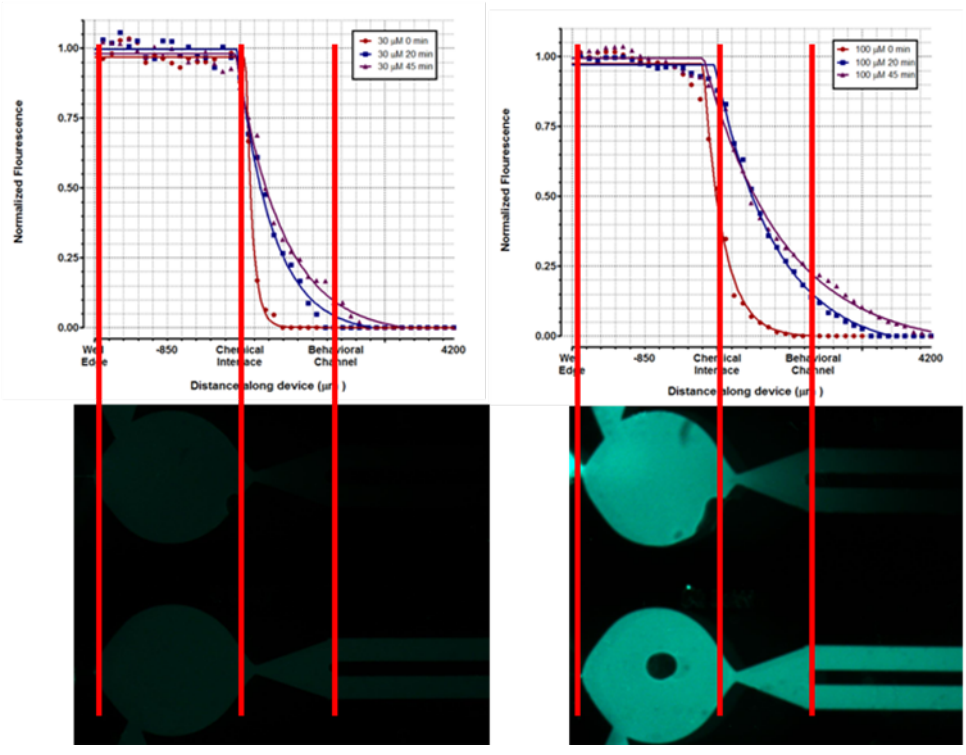
Theoretical work: SF3a shows theoretical diffusion calculations for priloclane in straight microfluidic channel. Both 30 µM and 100 µM concentrations are shown for times of 10, 20, 30 and 45 minutes. Priloclane was chosen because its diffusion is well studied and its molecular weight is representative of a worst case drug (i.e. is a very small molecule). In the above equation, c(x) is the drug concentration at a distance x from the point source, c_0 is the initial drug concentration, D is the diffusion coefficient and t is the time.

Experimental work: The experiments were conducted by filling the channels (with the two-step process described in the manuscript) and allowing the bioassay to sit untouched for 45 min. Images were acquired

every 5 minutes and ImageJ (NIH) software was used to track the amount of fluorescence as both a function of time and a function of distance along the channel. The raw data was modeled using a 'plateau followed by a single-phase decay' fit; all data sets showed a good fit with R squared >0.99.



SF3b Experimental diffusion of Fluorescein in 0.8% w/v agar/tap water. The raw data along the decay model for drug well concentrations of 30 µM and 100 µM are shown.

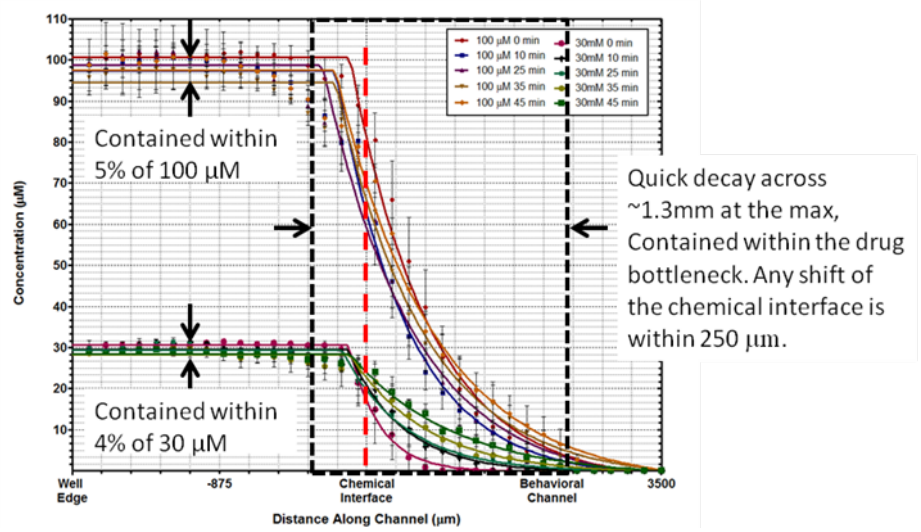


SF3c Diffusion raw data along the decay model (normalized to 1) as it compares to two images from which data was taken. Important boundaries are marked with red lines. Only diffusion is shown here with no electrically induced effects.

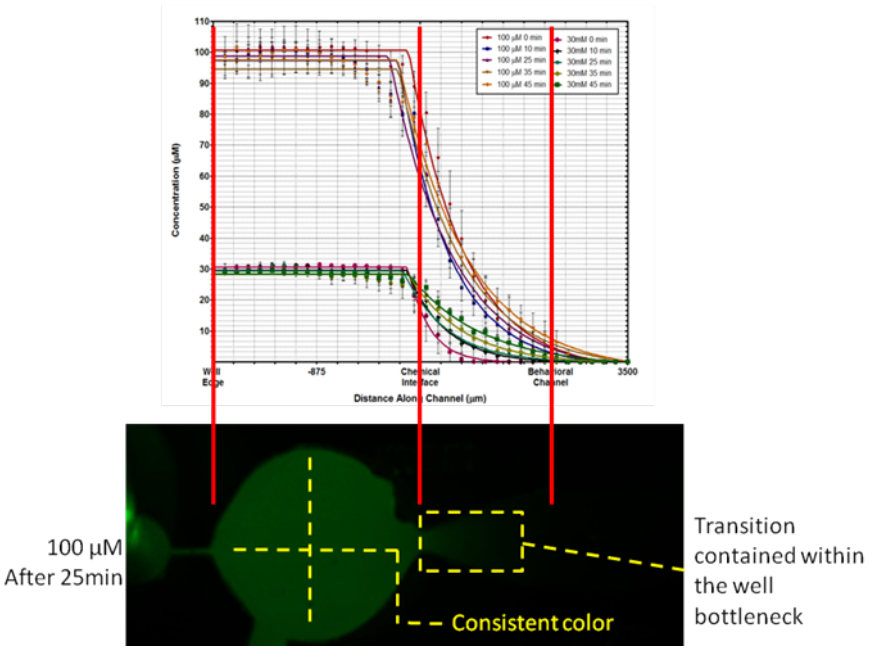
SF3b, c shows the experimental diffusion data along the decay model. As expected from the theoretical calculations (SF3a), the diffusion length is a function of time. One can see that after just 10 min the dye begins to move into the behavioral channel. Interestingly, in contrast to the theoretical calculations, the experiments showed diffusion length is also a function of concentration. Even at concentrations as high as 30 µM, diffusion is limited to a maximum of 1,000 µm into the neck after 45 min; as compared to theoretical calculations which predict a diffusion >4100 µm. This is encouraging as it shows that the slight diffusion (for concentrations up to 30 µM) is expected to be contained with the region between the drug well and the behavioral channels. At these concentrations (<30 µM) the dose in the drug well is kept within 2.4% (high=29.99 µM; low=29.28 µM for 30 µM well) of the intended dose, showing no signs of a dose gradient. The chemical interface (i.e. the start of the decay, X_0) is kept within 6% of the original value. The decay from X_0, reaches a half-length at x=239.45 µm (average) and fully dissipates by 800 µm into the tapered neck. Using these values, coupled with the velocity data presented in the manuscript, the average worms will receive only 13 seconds of 'extra' exposure (at a lower concentration of course) before reaching the well. This represents only 1.4% of the total exposure time. At higher concentrations (100 µM) diffusion will extend into the behavioral channels and worms may receive up to ~52s of extra exposure time. This represents a 5.7% increase (occurring at a lower concentration than the drug well) in total exposure time – bordering the significance line. This shows that when a neutral drug is to be used caution should be made as to the highest concentration to be tested. We will next show that when a Nicotinic (positively charged anthilmentic) is to be used, electrokinetic forces negate the diffusion effects – containing all movement within the tapered neck.

Electrokinetics

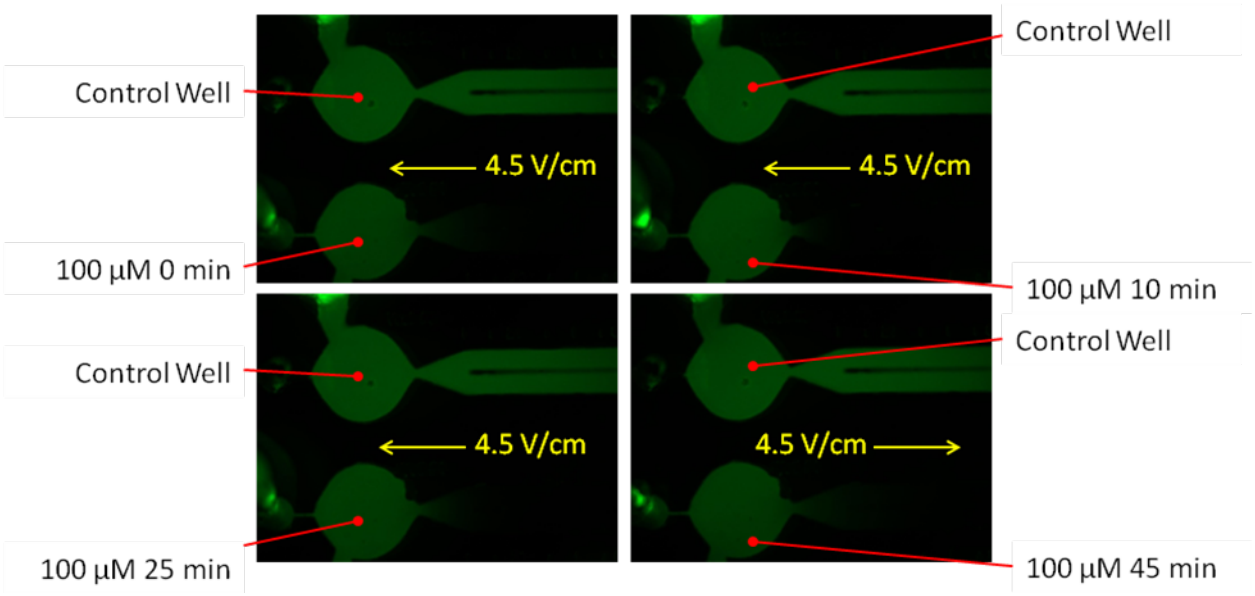
To mimic the electric force felt by a positively charge anthelmintic, an electric field of 2.25 V/cm was applied directed from the drug well toward the input port (you will notice this is reversed in direction and half the strength of the field used in the manuscript, this is to account for the opposite and double charge of the dye when compared to a representative anthelmintic). This field was held constant for 25min – 10 minutes to mimic the 'pre-exposure load time' and 15 minutes to mimic the 'exposure hold time'. The field direction was then reversed and held for 20 minutes to mimic the 'post-exposure unload time'.



SF3d Electrokinetics plus diffusion raw data along the decay model.



SF3e Electrokinetics raw data along the decay model as it compares to a sample picture from which data was taken. Important boundaries are marked with red lines.



SF3f Time-lapse images showing the progression of the Fluorescein dye through the electrokinetics experiments. One can see there is a slight diffusion directly after filling. During the next 25 min diffusion effects are nearly negated by electrophoresis and the transition from the drug well dose to the channel agar is confined to the tapered neck.

SF3d-f summarize the electrokinetics experiments, we note that these experiments do not isolate electro-induced effects, but also include diffusion and, thus, represent the net movement of a drug throughout the entire experiment. As shown SF3d, the addition of electro-induced effects further contains the diffusion (most importantly for 100 µM). The raw data along the decay model is shown here. One can readily see the electrokinetic effects – during the first 25 min the decay is pulled back and its slope steepens (10%

reduction in half-length). This is intuitive as the negative ions will be attracted to the anode. During the last 20 min (after the field is reversed) the decay relaxes slightly, nearing the 0 minute profile. The chemical interface remains stable within 250 μm of the 0 minute interface (a variation less than the length of an *O. dentatum* namatode). Most importantly the well concentration is kept within 5% (from top to bottom) of the desired dose (hitting a max of 101 μM and a low of 96 μM, we note there is also some compounding error in the analysis, thus we expect this to be a worst case number). The decay from the chemical interface falls rapidly and is well contained within the neck. A half-length is hit at 365 um (for a 100 μM well concentration) and 270 um (for a 30 μM well concentration) and the dose is dissipated by the end of the neck (1,000 um for 100 μM concentration and 900 um for 30 μM concentration). This shows that the average worms will receive a maximum of a 1.8% increase in exposure time.