An Easy Lab Exercise to Teach Fickian Diffusion, Mathematical Modeling, and Drug Delivery

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Statement of Purpose: Diffusion of molecules in hydrogels is an important concept in drug delivery, biomaterial design, and tissue engineering. The purpose of this laboratory exercise is to introduce the concepts of Fickian diffusion as a mechanism of drug release from hydrogels, as well as the dependence of the diffusion coefficient (D) on polymer-drug interactions, in a manner that is inexpensive, fast (less than 2hrs), and easy to do with large numbers of students. In addition, the utility of mathematical modeling in biomaterials characterization is demonstrated. Students, working in groups of 3-4, are given gelatin polymer (they are not told whether it is type A or type B) and solutions of methylene blue (MB) and fast green (FG) as model drugs. They prepare gelatin hydrogel thin films loaded with MB or FG and take samples of the release supernatant over 60min. Because MB is positively charged and FG is negatively charged at neutral pH, their relative rates of release will indicate whether the gelatin is positively charged (type A) or negatively charged (type B). The students fit curves to the data based on the Ritger-Peppas equation for one-dimensional release of hydrophilic drugs from thin hydrogel films, in order to determine D:

$$\frac{M_t}{M_\infty} = 4\left[\frac{D\,t}{\pi l^2}\right]^{1/2}$$ (Ritger, P. J Contr Rel 1987; 5:23-36)

where M_t is the mass of drug released at time t, M_∞ is the total mass of drug released at infinity (i.e., drug loading), and l is the thickness of the film. This equation is derived from Fick's second law of diffusion, and is valid for small values of time (valid for the first 60% of released drug).

The students use the value of D obtained from the equation to determine which type of gelatin they were given.

Methods: In order to ensure that release of the drugs from hydrogel films is one-dimensional, 50-ml conical tubes are filled at the bottom with poly(dimethyl siloxane) (PDMS) (Sylgard Elastomer kit, Fisher NC9897184, 1.1lb for $91.38, enough for ~100 preparations) so that the hydrogel can be formed as a thin layer on top of the PDMS layer (**Fig. 1a**). This should be done by the instructor before the lab session. Gelatin solutions (type A, Sigma G2500, $33.40 for 100g; type B, Sigma G6650, $32.20 for 100g) (9wt% in PBS) should also be prepared before class and maintained at 37°C to avoid premature gelation. Solutions (5mM) of MB (Sigma M9140, $61.40 for 25g, sufficient for 4,464 preparations) and FG (Sigma F7252, $46.20 for 5g, sufficient for 417 preparations) should also be prepared before class. Students mix the gelatin solution and one of the drug solutions in equal parts, and add 1ml to the conical tubes. Gelation is allowed to occur over 15min with the tubes on ice. Then, 20ml PBS is added to each tube containing hydrogels with each drug, and diffusion is allowed to occur over 60min. Students should observe the drug leaving the gel layer and entering the PBS. Every 5-10min, the students should swirl the tubes to ensure homogenous solution and take an aliquot of 150ul from the release supernatant. The samples are added directly to a clear 96-well plate (such as Fisher 12-565-226, case of 60 for $151.70). Students should also prepare standard curves of both MB and FG by serially diluting 5mM to 0.0025mM. Absorbance values of the release samples and the standard curves are obtained using a plate reader (peak absorbance is 664nm for MB and 625nm for FG). The data is emailed to the class, compiled with other groups as experimental replicates, allowing statistical analysis of the release of MB and FG. The students convert the values of absorbance to concentration using the standard curve, and calculate the thickness of the gel using the diameter of a centrifuge tube (28mm) and the volume of hydrogel (1ml). They then fit the Ritger-Peppas equation through the data using MATLAB, or by plotting normalized concentration vs. the square root of time, for a linear fit. Alternatively, D can be determined using least squares regression in Excel using Solver. With any of these methods, the equation is used to calculate D for MB and FG, repeated for each replicate. Student's t-test is used to compare D for MB and FG.

Results: Students will find that this equation fits the data well and can be used to calculate D (**Fig. 1b**). D is higher for MB than FG when type B gelatin is used, or lower if type A gelatin is used. This lab exercise can be completed in a two hour lab session. The total cost of reagents is less than $5 per group (assuming each group uses one 50-ml tube each for MB and FG release and one 96-well plate).

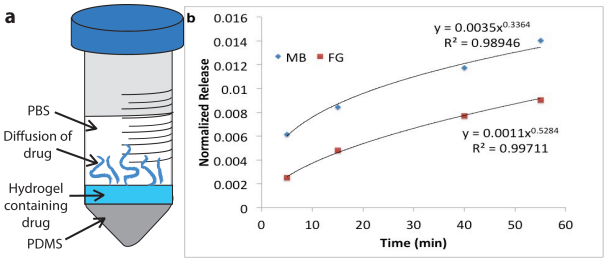

Figure 1. (a) Schematic describing how hydrogel thin films are prepared with one-dimensional diffusion by filling the bottoms of 50-ml conical tubes with PDMS. (b) Release of MB and FG from gelatin hydrogel thin films with curves corresponding to Fickian diffusion.

Conclusions: This lab can be used to teach important concepts in drug delivery, including Fickian diffusion and the role of polymer-drug interactions in determining the diffusion coefficient and release rate. In addition, students learn important skills such as the preparation and use of a standard curve, mathematical modeling, and simple statistics. We have each student write a conference-style abstract describing their results, and include a derivation of the Ritgers-Peppas equation from Fick's second law as an appendix. This real world application reinforces the calculus and differential equations they learned in earlier classes.