Barrier-Mediated Pulsatile Release

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Abstract.

A membrane-based approach to pulsatile delivery, termed here as Barrier-Mediated Pulsatile Release (BMPR), sequesters each chemical dose into its own stimuli-sensitive reservoir film, each covered by a stimulant barrier membrane to delay triggering for a prescribed period time. These barrier/depot pairs are then stacked sequentially, with the delay time for the next barrier commencing with the stimulation of the previous depot, assuring a controlled period between each pulse. This paper introduces the first generalized BMPR system, using hydrogel depots that can be adapted to a variety of stimulants, coupled with barriers that rely on sacrificial stimulant scavengers to provide controlled delay times spanning orders of magnitude. Poly(methyl methacrylate-co-dimethylamino ethyl methacrylate) hydrogels are used to demonstrate pulsatile release of multiple solutes triggered by buffered citric acid. Zinc oxide nanoparticles loaded in poly(vinyl alcohol) hydrogel barriers delay acid permeation to each depot. Depot thickness, pH, and buffer strength are each shown to affect the stimulant permeation, swelling, and solute diffusion rates from the depots, with different processes becoming rate-limiting under different conditions. The delay time for each barrier varies linearly with ZnO scavenger loading, scavenger center-of-mass, and the square of the barrier thickness, providing multiple pathways for tuning delay time over a wide range. BMPR devices releasing up to 10 pulses are demonstrated, with no inherent limit on the number of pulses possible.

1. Introduction

Many chemicals are most effectively delivered in discrete pulses rather than at a steady rate. Decreased chemical costs and environmental burden are one obvious advantage to only releasing chemicals when actually needed, be they herbicides in fields or biocides on ship hulls. Second, many biological molecules such as hormones are only effective when present in periodic pulses.[1] Vaccines,[2] cancer therapies,[3] and allergen treatments[4] often require repeated pulses in order to most effectively trigger desired biological responses, while other solutes such as nitroglycerin[5] and opiates[6] prompt tolerance within the body, quickly losing effectiveness at constant concentration. Third, many solutes best operate in sequential combinations. For example, one chemical may hinder insects' resistance mechanisms prior to exposure to a second chemical, an insecticide.[7]

Traditional means of achieving pulsatile solute release (manual spreading, spraying, swallowing, or injecting), however, are often impractical. For instance, pills and injections are difficult to administer while asleep. Such administration can be automated with sensors, timers and pumps, though such complexity can quickly become too expensive or unreliable for the application. A delayed pulse can also be achieved by encapsulating an individual dose behind a barrier that degrades or ruptures after a fixed period of time. In theory, one could use an ensemble of such capsules, each with a different degradation time, to achieve periodic pulsatile delivery in a single administration.[8, 9] In practice, however, this approach has never been demonstrated beyond three pulses. Since the timing of each capsule is determined independently, small timing errors can soon result in long delays punctuated by overlapping pulses, to say nothing of pulse-smearing within a given capsule set, nor the difficulty in tuning the capsule delay over multiple orders of magnitude.

This problem can be mitigated, however, by coupling the release of one pulse to the release of the previous one. In this approach, individual doses of solute are interlaced between barriers which delay release only for the additional time to the next pulse. A simple example is depicted in Figure 1, where individual depots of dissolvable chemical are separated by barrier layers of degradable polymer. Upon exposure to water the top polymer barrier erodes, eventually exposing the underlying drug depot which is promptly dissolved and released. At this point water reaches the next polymer barrier layer, eroding it over a similar period of time. It then reaches the next drug depot, releasing a second pulse of drug and triggering the erosion of the third polymer barrier, and so on.

Figure 1: Schematic demonstration of pulsed release from erodible polymer laminate. Upon immersion in water, the first solute pulse is released, followed by a period of no release while the top barrier layer erodes. Once the top barrier layer is gone, a second solute pulse is released, followed by erosion of the next barrier layer.

This approach, which we term Barrier-Mediated Pulsatile Release (BMPR), has been proposed previously,[10-13] but few examples of implementation exist, in part because the above example requires each barrier to be perfectly surface eroding. If any part of the barrier is breached before the rest of it, the breach will release some of the chemical prematurely and broaden the pulse. Also, the delay time of each barrier scales, at best, linearly with the barrier thickness.[14] As the bulk- vs. surface-erosion behavior of polymers also varies with the barrier thickness,[15] time-scaling of the barriers also becomes problematic. Here we report on our efforts to generalize the BMPR approach. By decoupling the delay time of the barriers from

polymer degradation and triggering the release of solute using stimuli other than water, simple laminates releasing up to ten pre-programmed pulses are demonstrated. Different solutes are released in alternating pulses, and the timing of each pulse can be scaled easily over a wide range.

To generalize the BMPR approach, solid soluble chemical is confined within a stimuli-sensitive hydrogel. These polymer networks swell with water in response to a stimulant, increasing their permeability dramatically. A wide variety of hydrogel stimulants have been demonstrated, including temperature,[16] pH,[17] glucose,[18] light,[19] electromagnetic fields,[20] and specific antigens.[21] This study employed pH-sensitive methacrylate hydrogels which are glassy at pH 7 but swell with five times their weight in water when subjected to acid at pH 3, as shown in Figure 2. Zinc oxide nanoparticles were used as a sacrificial scavenger within reactive barrier membranes to protect the acid-sensitive depot layers from the acid stimulant for a prescribed period of time. After exhausting the ZnO, the acid triggers the underlying depot layer to swell, delaminating it and its overlying barrier membrane while releasing its payload. The next barrier membrane is then exposed directly to the stimulant and the process repeats.

As the release is decoupled from polymer degradation, the barriers and depots must degrade on a time scale much slower than the chemical release, which may be problematic for some applications. This delamination also adds an additional failure mode which must be addressed for each application. Stimulation by pH, however, opens this approach to many other potential stimulants such as hydrocarbons, which can be converted to organic acids *in situ*. Bacterial biofilms in oil tanks, for instance, generate acids which are harmful to downstream equipment. Individual biocide pulses could automatically mitigate these biofilms when their acid generation becomes problematic.

2. Materials & Methods

2.1 Materials

Methyl methacrylate (MMA), 2-dimethylamino ethyl methacrylate (DMA), and divinyl benzene (DVB) were obtained from Aldrich (Milwaukee, WI). Polymerization inhibitors in these monomers were removed by adsorption on

Figure 2: Poly(methyl methacrylate-co-dimethylamino ethyl methacrylate) disk at pH=7 (left) and pH=3 (right). Under neutral conditions the polymer is glassy and hydrophobic, effective encapsulating water-soluble drug powders. Under acidic conditions the polymer swells with water, dissolving and releasing drug. Aqueous blue dye was added for contrast.

alumina (Fisher Scientific, Pittsburgh, PA), the monomers were then refrigerated until used. 2, 2'-Azobisisobutyronitrile (AIBN), zinc oxide (ZnO), citric acid, sodium hydroxide, and potassium chloride were obtained from Sigma-Aldrich and used as received. Monobasic potassium phosphate and sodium chloride were used as received from Research Products International (Mt. Prospect, IL). Poly (vinyl alcohol) (PVA, 99% hydrolyzed, MW ~ 133,000) was obtained from Polysciences (Warrington, PA) and used as received. Methylene blue (MB, Sigma-Aldrich) and methyl orange (MO, Alfa-Aesar, Wardhill, MA) were ground with a mortar and pestle then screened at 100 mesh before use.

2.2 Device fabrication

2.2.1 Depot layer fabrication

Poly (methyl methacrylate) (PMMA) is a well-studied polymer with excellent mechanical properties and a wide range of commercial applications. Incorporation of acid-sensitive comonomers into PMMA has been previously studied, with dimethylamino ethyl methacrylate (DMA) in a 70/30 (by mol) MMA/DMA ratio retaining both the glassy, hydrophobic character of PMMA at neutral pH and showing a strongly hydrophilic swelling behavior at pH 6 and

below.[17, 22, 23] This impermeable “off-state” in the absence of stimulus is a critical feature for BMPR depot materials, as it allows large doses of solid soluble chemical to be loaded into each depot and retained for long periods of time without significant degradation or leakage. It also complicates control of the depot swelling rate, however, as discussed in Section 3. By incorporating a co-monomer or cross-linking agent which is hydrolytically or enzymatically cleaved, biodegradable pH-sensitive PMMA gels have also been produced.[24]

To create chemical-loaded poly (methyl methacrylate-co-dimethylamino ethyl methacrylate) (PMMA/DMA) films, 3.909 mL of DMA was added to 6.315 mL MMA, 10.5 μ L divinyl benzene crosslinker, and 48.03 mg 2,2'-azobisisobutyronitrile (AIBN) in a 20 mL vial. After heating to 70 °C with stirring, the solution polymerized to a viscosity of approximately 65 cP before being chilled to room temperature. Methylene blue (MB) powder (a model drug released in these studies) was then dispersed into the oligomer solution by stirring and sonication (Cole-Parmer CPX750 with 3.2 mm microtip, 1 min at 300 W, 20 kHz). The suspension was then poured between two silanized glass plates, with poly (tetrafluorethylene) (PTFE) spacers clamped between them. Different spacers were used to make films of different thicknesses from 50 to 350 μ m. The plates were then mounted vertically in a vacuum oven at 60 °C under nitrogen for 18 hours, after which the MB-loaded polymer film was cooled, removed from the glass plates, and stored under ambient conditions until used. Thickness variation across an 18-cm wide film could exceed 20 μ m due to the negative volume of polymerization in this closed system, though individual depots cut with a 15-mm-wide cork borer had thickness variation of less than 2 μ m.

2.2.2 Barrier layer fabrication

Reactive acid barrier membranes using zinc oxide dispersions in poly (vinyl alcohol) (PVA) have been described previously.[25, 26] Briefly, 15 g PVA were dissolved in 135 mL water at 90 °C under stirring. Zinc oxide nanoparticles corresponding to the desired dry weight percentage (10-40%) were added and dispersed through stirring and sonication. The large surface area of the ZnO nanoparticles assured rapid scavenging of any passing stimulant acid, as well as homogenous distribution of the ZnO even at very thin film thicknesses. The suspension was then cooled to room temperature and cast on a PTFE sheet, followed by calendaring with a custom-built doctor blade to the desired thickness. After drying for at least 24 hours under ambient conditions, the films were peeled off, clamped between two glass plates, and cured at 150 °C under vacuum for two hours to crosslink. Films were stored under ambient conditions then immersed in water to hydrate at least 24 hours before use. After hydration, individual barriers with a thickness variation of less than 3 μm were cut using a 16-mm-wide cork borer. Barrier-to-barrier thickness variation from a given 10-cm-wide coating was larger, however, due to a variety of issues associated with calendaring viscous suspensions.

2.2.3 Device fabrication

Disks of dry PMMA/DMA depot film 15 mm in diameter were stacked alternately with 16 mm diameter disks of hydrated PVA barrier film atop a 0.5 mm thick, 16 mm diameter PTFE plug. The plug was placed within a circular PTFE mold 6.7 mm thick and 135 °C beeswax was poured into the mold, surrounding and covering the polymer stack. After freezing for 15 min, the PTFE plug was removed, leaving a simple coin-shaped laminate pulsatile release device.

The primary challenge in device fabrication is reliably achieving complete contact between the wax and every hydrated barrier layer, to avoid stimulant leakage around the edge of the barrier. Besides making the barrier layers slightly wider than the depots and recessing the

stack slightly into the wax sidewall using the PTFE plug, a hydrophobic PMMA/DMA layer was placed on the outer face of every device, even when an immediate solute pulse was not desired. In those instances, this outer depot layer contained no payload and effectively acted as simply another barrier layer.

2.3 Device characterization

2.3.1 Depot layer characterization

To confirm the dimethyl amino ethyl methacrylate (DMA) content of the depot membranes, dry samples (40 mg average weight) were individually equilibrated in 150 mM saline solutions (~300 times sample weight) initially set to pH 2 via HCl addition. Upon addition of the polymer sample, the pH of each solution rose to 2.76 ($\pm$ 0.06 on $n = 3$). At equilibrium, all amine groups in this polymer are protonated in this pH range,[27] allowing the MMA:DMA mol ratio in the depot membranes to be stoichiometrically calculated as 75:25.

The swelling rate and equilibrium swelling ratio of drug-free PMMA/DMA membranes were characterized against three parameters: pH, membrane thickness, and buffer concentration (neutral weak acid + conjugate base). Circular disks (12 mm diameter) of PMMA/DMA membrane were immersed in 250 mL buffer and periodically reweighed.

For pH-dependency trials, 223 μm ($\pm$ 1.7% RSD) thick membranes were immersed in citrate (pH 3, 4, 5 or 6) or phosphate (pH 7 or 8) buffer at a buffer concentration of 0.01 M. All buffers used a sodium counter-ion and were set to an ionic strength of 0.1 M by NaCl addition unless otherwise indicated. To investigate the effect of membrane thickness on swelling rate and equilibrium swelling ratio, membranes ranging in thickness from 110 to 350 μm were tested at pH 3. These trials were performed at buffer concentrations of both 0.01 M and 0.1 M to demonstrate the effect of buffer concentration. All trials were performed in triplicate.

Additional swelling information was gained in conjunction with release rate trials using PMMA/DMA disks loaded with 5% (w/w) methylene blue powder. For these trials, depots ranging in thickness from 75 to 350 μm were tested first in pH 7 buffer, then transferred to 0.01 M pH 3 buffer. Swelling was measured gravimetrically as above, while release of methylene blue was measured by a UV/Vis spectrophotometer (Varian Cary 50, Mulgrave, Australia) equipped with an immersion probe.

The transport properties of both the depot and barrier membranes were measured using a diaphragm cell. Briefly, the film was sandwiched between two well-stirred 22 mL cups. The upstream cup was spiked with solute and the downstream cup's solute concentration was measured vs. time to determine a steady-state flux, from which the membrane's permeability was calculated. For methylene blue permeability trials, depot films were soaked in 0.01 M buffer at pH 3 or pH 7 for 1-3 days prior to testing. Additional trials doubling the stirring speed in the diaphragm cell reservoirs confirmed negligible mass transfer resistance between the membrane and the adjacent reservoir liquids.

Acid transport across the depot membrane is inherently more difficult to characterize, in part because the membrane's transport properties are pH-dependent. For these studies, a titrator (Metrohm Titrando 842, Herisau, Switzerland) maintained the downstream pH at 4.0 (initial citrate buffer strength 0.001 M) while recording the rate of sodium hydroxide addition required to maintain this pH across from an upstream citrate buffer (initial buffer strength 0.01 M) at pH 3. The rate of NaOH addition to maintain constant downstream pH indicated the rate of total acid transport across the membrane. Prior to testing, the membranes were soaked in pH 4 citrate buffer (buffer strength 0.001 M) for three days, making their initial acid concentration comparable to the downstream reservoir.

2.3.2 Barrier Layer Characterization

To confirm that the ZnO content of the barrier membranes matched their recipe, 20 mg (dry weight) samples were individually placed in 100 mL of 0.001 M HCl. As the ZnO in the membrane consumed the acid, additional 0.001 M HCl was metered into each container to maintain a target pH of 3.5 using an automatic titrator. The difference between the final hydronium ion content and the initial and added hydronium content indicated the amount of ZnO in the sample, with each ZnO unit consuming two hydronium ions. The average discrepancy in ZnO weight fraction between the fabrication recipe and subsequent titration was 0.3% across three separate membranes.

This demonstrated that the ZnO concentration of each dry barrier membrane can be precisely controlled by its fabrication recipe, however it is the ZnO concentration in the barrier membrane's swollen state that determines its performance. To characterize barrier membrane swelling, dry barrier membranes of various ZnO weight fraction (0 – 80%) and thickness (39 to 167 μm) were swollen in deionized water for several days, then reweighed.

To confirm that the ZnO did not settle to one face of the barrier membrane during fabrication, membrane cross-sections were inspected by secondary electron microscopy. Figure 3 shows SEM images of a PVA film with no ZnO (A), a PVA film containing 10% (dry weight basis) ZnO (B), and a PVA film in which the ZnO is distributed through only half the PVA thickness (C). The latter film was obtained by casting a ZnO-free PVA layer atop a previously cast and dried ZnO-laden PVA layer, prior to cross-linking. While the SEM images indicate qualitatively the homogenous dispersion of the ZnO, slight asymmetry in the ZnO distribution (i.e., settling) is more difficult to detect with this method. Such asymmetry will, however,

noticeably alter the lag time of acid across the film, with faster acid breakthrough occurring if the scavenger has settled toward the upstream face of the membrane.[26]

Figure 3: SEM cross-sectional images of PVA barrier films. (A) without ZnO scavenger, (B) with 10% (w/w) ZnO scavenger, (C) as a bilayer with no ZnO in the upper-left and 10% (w/w) ZnO in the lower right (dashed line added at bilayer interface).

To test for such asymmetry, as well as measure the transport properties of the barrier membranes, diaphragm cells studies as described for the depot membranes were also performed on the barrier membranes. These experiments used an upstream 0.01 M pH 3 citrate buffer with a downstream solution of 100 mM NaCl at neutral pH. Membranes with initial ZnO weight fractions (dry basis) ranging from 0 to 60% were tested, with replicates oriented in opposite directions to detect any settling of the ZnO. The barriers ranged in thickness from 72 to 520 μm, with the thickest barriers comprised of multiple layers of thinner membranes in series. Select ZnO-laden films were soaked in deionized water for at least three days after testing, then retested to observed their acid lag-time and permeability after removal of the ZnO. Due to the high acid scavenger content of some barriers, the upstream reservoir was titrated as needed with 0.1 M HCl to maintain its pH at 3. Further diaphragm cell studies determined the permeability of methylene blue and methyl orange across ZnO-free PVA films ranging in thickness from 92 to 373 μm. These trials used 0.01 M pH 3 citrate buffer on both sides, with the target solute spiked upstream.

2.4. Device testing

The release behavior of each device was determined by taping it to a glass platform and immersing it in >0.3 L of 0.01 M buffer. Phosphate (pH 7) and citrate (pH 3) buffers were used for these trials, with the pH monitored by pH meter and the UV/Vis spectrum of the buffer solution periodically scanned using an immersed fiber-optic probe coupled to a UV/Vis spectrophotometer. Pictures of the buffer and immersed device were recorded every 15 minutes by a PC-interfaced camera. Buffer solution was added and/or replaced as needed to keep the optical absorbance of relevant wavelengths below 1.0, also ensuring that the buffer composition did not drift significantly.

3. Results and Discussion

3.1. Depot layers

One critical feature of BMPR depots is an “off state” in which the chemical release rate is negligibly small. This criterion immediately eliminates some of the most popular stimuli-sensitive polymers, most notably acrylamide copolymers, which even in their shrunken

Figure 4: Solute Release from PMMA/DMA hydrogels. No release at pH 7 except for small (~4%) initial burst due to film cutting. Prompt, complete release at pH 3.

hydrophobic state are rubbery and contain a non-negligible amount of water. Figure 4 demonstrates that PMMA/DMA hydrogels meet this criteria. Their extreme hydrophobicity and glassy structure at neutral pH effectively block the dissolution and diffusion of all encapsulated drug; only ~4% of the solute was released at pH 7, and that drug was released instantly, likely because it was directly exposed to the buffer solution around the perimeter of the disk where it was cut. A second critical feature is a highly permeable “on state” in which the chemical can

rapidly dissolve and diffuse away. This is also shown in Figure 4, where at pH 3 nearly all of the drug in the thinner depots is released in the first hour of exposure, despite a day without release at pH 7.

The depot swelling trials indicate that the swelling is strongly pH-dependent and, in most cases, transport-limited. This is in good agreement with previous studies of PMMA/DMA hydrogels.[22, 28] The sigmoidal shape of the swelling curves in Figure 5 (shown more clearly in the inset)

Figure 5: Swelling of PMMA/DMA hydrogels. Equilibrium swelling decreases with increasing pH and appears negligible at pH 7. Error bars show standard deviation with n=3. Sigmoidal shape of swelling curve at short times (inset) shows that swelling rate is transport-limited.

indicate a swelling front advancing from each face of the membrane towards the center. As the fronts move deeper into the membrane, the rate of acid diffusing to the front to advance it decreases, slowing its progress and hence the swelling rate. When the fronts meet at the center of the membrane, however, swelling is no longer confined to one dimension and the membrane rapidly swells in the other two dimensions before reaching equilibrium. Figure 6 further demonstrates that the swelling is pH-dependent and transport limited by comparing swelling rates at pH 3 and 7 for depots of different thickness. None of the membranes swell at all at pH 7 and all membranes swell rapidly at pH 3. Moreover, all but the thinnest membranes show sigmoidal swelling curves as well as swelling rates that vary with the square of the membrane thickness. Notably, the thinnest membranes (112 μm average) do not have a discernable sigmoidal shape to their swelling curve, and their overall swelling time is not four times shorter

than the 230 μm membranes', despite being twice as thin. The swelling still takes roughly 30 minutes, suggesting that this is a polymer relaxation limit, consistent with earlier studies.[28]

While Figures 5 and 6 demonstrate that the swelling rate is pH-dependent and diffusion-limited, Figure 7 indicates that this is not due to larger H^+ concentrations

driving faster H^+ diffusion. Rather, at uniform buffer strength the concentration of neutral weak acid increases strongly with decreasing pH, and it is diffusion of this neutral weak acid that controls the swelling rate. When the buffer strength is increased from 0.01 M to 0.1 M at the same pH (3), the swelling rate increases dramatically. This effect has been explored

Figure 6: Thickness dependence of swelling P(MMA/DMA) hydrogels. All three pH 7 lines overlay each other with no significant swelling. At pH 3 swelling rate varies with film thickness. Note inflection in swelling curves of films more than 150 μm thick.

Figure 7: Effect of buffer strength and film thickness on swelling PMMA/DMA hydrogels. At any thickness, films swell significantly faster in more concentrated buffer at the same pH, underscoring the importance of the neutral weak acid in these systems.

previously[27] and can be explained by the strong hydrophobicity of the shrunken gel. While H^+ can diffuse very rapidly in aqueous media, it cannot penetrate into the hydrophobic region of the polymer and is therefore limited to protonating the amines right at the interface of the hydrophobic region, advancing that interface very slowly. By contrast, a neutral organic acid can partition into the hydrophobic region, then dissociate when it encounters an amine. Being able to protonate amines beyond the hydrophobic interface, it can advance that interface much more rapidly. This was most starkly demonstrated by swelling trials in HCl at pH 3, where the swelling time was measured in days rather than minutes. The 0.1 M buffer trials in Figure 7 also further demonstrate the second-order dependence of swelling on membrane thickness. Moreover, even the thinnest membranes swell faster at a higher buffer strength, indicating that diffusion is still significant, though not completely rate-limiting, under those conditions.

This has significant implications for adapting BMPR systems with pH-sensitive hydrogels to specific applications. For instance, while the digestive tract maintains a strongly acidic environment, gastric acid is unlikely to be an effective stimulant for this sort of hydrogel (assuming all other barriers to digestive tract implementation were overcome). Enzymatic conversion of blood glucose to gluconic acid, on the other hand, may provide a viable stimulant, despite external pH buffering by the blood. The presence of a particular external pH is relevant only if it influences the flux of neutral acid species that can be delivered, an influence that varies with the pK_a of the acid and the location at which the acid is generated.

The neutral acid flux also plays a prominent role in the diaphragm cell transport studies. As discussed in the Supplemental Information, its diffusion coefficient is generally smaller than that of dissociated protons, but its concentration gradient from 0.01 M pH 3 citrate buffer is

larger, resulting in comparable fluxes of dissociated and neutral acid. By titration only their combined flux is measured, at 1.1×10^{-9} mol/(cm² s) for 500 μ m of membrane, scaling inversely with membrane thickness. Since the two species have different concentration gradients, a meaningful permeability cannot be directly calculated. By comparison, however, the combined flux across a hypothetical 500 μ m membrane of pure water between those reservoirs would be 1.3×10^{-9} mol/(cm² s), only 20% higher. That hypothetical pure water membrane would also have 20% more water than the hydrogel membrane, as the polymer occupies 15-20% of the membrane volume at equilibrium in those conditions, as indicated in Figure 5. In short, the acid species appear to diffuse across the swollen depot membrane with the same diffusion coefficients as in water and with partition coefficients equal to the hydrogel's water volume fraction.

While the transport coefficients for small stimulant molecules through the swollen depot may be estimated from their coefficients in water, the diaphragm cells studies on methylene blue and methyl orange yield a different result. Here the depot films were sandwiched between 0.01 M pH 3 buffer on both sides, with a spike of dissolved drug added to the upstream cell. The permeabilities of the methylene blue and methyl orange through PMMA/DMA were 3.4×10^{-7} cm²/s and 3.0×10^{-7} cm²/s, respectively. Using the lag times of these trials, the diffusion coefficient of methylene blue in the depot film was calculated at 7.1×10^{-7} cm²/s, yielding a partition coefficient of 0.43. This was confirmed by equilibrating depot films in a pH 3 buffer of known methylene blue concentration, then desorbing the solute into solute-free buffer for quantification, yielding a partition coefficient of 0.48. Unlike acid, methylene blue avoids entering the depot (partition coefficient less than the gel's water volume fraction) and diffuses more slowly in the gel than it does in water ($\sim 7 \times 10^{-6}$ cm²/s),[29] perhaps owing to its larger size (MW 320 g/mol), planar configuration, and positive charge. Since the depot will serve as an ion

exchange resin for the negatively charged methyl orange, the distinction between partitioning and adsorption becomes blurred. Its low permeability, however, suggests that it also diffuses more slowly in the gel than in water. Its size (MW 327 g/mol) and configuration (planar) are comparable to methylene blue's. No measurable solute transport was observed in diaphragm cell studies at pH 7 for either solute.

Despite these slower mass-transport coefficients, the release rate of chemical leaving the depot appears to be on par with the swelling rate, as shown in Figure 8 which plots both swelling and chemical release vs. time for depots of different thicknesses. Besides noting that the equilibrium swelling ratio for methylene blue loaded depots is higher than for the drug-free depots of Figure 5 (methylene blue inhibits polymerization,[30] likely prompting a looser network), Figure 8 also shows that the time required for the depot to release its payload is approximately twice its swelling time regardless of the film thickness, indicating that solute release is also transport limited.

Figure 8: Comparison of swelling and solute release rates in PMMA/DMA hydrogels. No swelling and negligible release of methylene blue at pH 7. At pH 3 the characteristic swelling and release times can be estimated analytically.

This suggests that one can adapt Higuchi's transport equations[31, 32] to estimate characteristic times for both swelling (t_{swell}) and chemical release (t_{rel}):

$$t_{\text{swell}} = \frac{C_{\text{scav}}}{C_{\text{stim}}} \frac{\ell^2}{2 DH_{\text{stim}}} \quad t_{\text{rel}} = \frac{C_{\text{solid}}}{C_{\text{sat}}} \frac{\ell^2}{2 DH_{\text{solute}}} \quad (1, 2)$$

C_{stim} and C_{sat} are the external acid concentration and the drug saturation concentration, respectively, while C_{scav} and C_{solid} are the concentration of acid-scavenging amine groups and solid chemical in the depot, respectively. DH is the permeability of the indicated species in the depot, and ℓ is the overall distance across which transport must occur. For a depot with homogeneously dispersed chemical, release starts at the same time as swelling, but clearly cannot finish before the swelling is complete, so $t_{\text{rel}} < t_{\text{swell}}$ simply means that the release is limited by the depot's swelling rate. For $t_{\text{rel}} > t_{\text{swell}}$, however, release will continue significantly after the depot has swollen and delaminated, while stimulant is penetrating the next barrier membrane. If $t_{\text{rel}} - t_{\text{swell}}$ is longer than the lag time of the next barrier, the next pulse may begin before the previous pulse has finished, resulting in continuous release if the chemicals in both depots are the same.

For the depot membranes in Figure 8, ℓ is the half-thickness of the membrane in the swollen state. While this transport is occurring the swelling is constrained to one dimension by the not-yet-swollen center of the film. From the sigmoidal curves in Figures 5-8 we see that the membranes swell by a factor of two before the one-dimensional constraint is lifted and the membrane swells more rapidly. This is verified by PMMA/DMA membranes covalently bound to glass substrates to permanently restrict swelling to one dimension. At equilibrium in the same pH 3 citrate buffer they double in both size and thickness. Doubling the half-thickness of each membrane in Figure 8, ℓ ends up being equal to the dry thicknesses listed in the figure legend. The amine concentration and solid drug concentration for those membranes under those

conditions are 1.17 M and 0.073 M, respectively, and the methylene blue saturation concentration determined in Section 2 is 5.9 mM. Using the permeabilities and acid concentrations discussed earlier, t_{swell} for depot membranes with dry thicknesses of 75, 150, and 260 μm are 0.17, 0.66, and 1.99 hours, respectively, matching the curves in Figure 8 quite well. The characteristic release times for these membranes are comparable: 0.28, 1.14, and 3.41 hrs, respectively, indicating that both rate processes influence the final release profile in these release trials. To ensure that the release from sequential depots is pulsatile, the lag time of the intervening barrier membrane must be comparable.

3.2 Barrier Layers

Ideally, the barrier layer blocks stimulant from reaching the depot layer for a fixed period of time, then allows the stimulant into the depot unhindered. To achieve this ideal, the barrier must have a large innate permeance to the stimulant while simultaneously having a large stimulant-scavenging capacity. The characteristic lag time (t_L) for stimulant permeation across a barrier loaded with a sacrificial scavenger is [26, 31, 33]

$$t_L = \frac{l^2}{6D} \left(1 + \frac{6 C_{\text{scav}} Z_m}{v H C_{\text{stim}}} \right) \quad (3)$$

where C_{scav} is now the concentration of acid-scavenging ZnO groups (rather than amine groups, as in the depot layer) and v is a stoichiometric coefficient (in this case, $\frac{1}{2}$ since $\frac{1}{2}$ of a ZnO scavenger consumes a proton). Z_m is the location of the scavenger's center of mass across the film; for homogeneously distributed scavenger, the center of mass is midway across the film and $Z_m = 0.5$. Equation 3 is essentially a more precise version of Equation 1 which accounts for heterogeneity in scavenger distribution and the tiny amount of stimulant still needed when C_{scav} approaches zero. Like Equation 1 it shows that the lag time varies with both the concentration of scavenger and with the square of the film thickness. These relationships are demonstrated in

Figure 9, where breakthrough curves of integrated acid flux vs. time are plotted for PVA films with ZnO content ranging from 0 to 30% (dry weight basis) and thicknesses ranging from 72 to 224 μm . The lag time for ZnO-free films is on the order of a minute, while a 94- μm -thick film with 10% ZnO by weight has a lag time of 1.64 hrs. Based on Equation 3, the lag times for the 20% and 30% ZnO films should be larger by the ratio of ZnO fractions times the square of the ratio of film thicknesses, to 1.92 and 3.99 hrs, respectively. Experimentally, these films had lag times of 1.86 and 3.97 hrs, respectively. Stacking three 20% ZnO films together, the lag time increases to 15.63 hrs, within 7% of the expected factor of 3^2 increase.

Figure 9: Breakthrough curves for acid across barrier films. Lag time prior to acid breakthrough increases linearly with ZnO concentration and with the square of film thickness. Acid permeance, as indicated by the breakthrough slope, is independent of ZnO loading and varies only inversely with barrier thickness.

While the lag time increases by an order of magnitude, however, the permeability remains unchanged. Diaphragm cell trials indicate an acid permeability across the barrier membranes of $1.47 (\pm 0.1) \times 10^{-5} \text{ cm}^2/\text{s}$. As PVA has little pH sensitivity, acid flux in these trials was measured directly from pH shift in the unbuffered downstream reservoir, spanning into a pH range where the flux of neutral acid species contributes little (~12%) to the measured shift. This

was confirmed by trials using unbuffered citric acid to operate in a lower pH range where even less of the neutral acid flux would dissociate downstream; under those conditions the observed permeability was only 9% lower. The importance of this result is to demonstrate that despite being able to completely block the stimulant for a long, prescribed period of time, the barrier membrane still has a very large stimulant permeability, comparable to pure water. Swelling studies demonstrated that the membrane (excluding the scavenger) is 55% water by volume. The acid permeability listed above is 46% of the acid permeability through pure water. This suggests that, like the depot membrane, acid permeates the barrier membrane nearly as it would through pure water, unaffected by the surrounding polymer superstructure. Moreover, initial incorporation of ZnO should have little effect on permeability, both because the volume of the ZnO is so small (<10% by volume for a membrane initially containing 50% (dry weight basis) ZnO) and because once the ZnO is consumed that volume is filled by a medium (water) with a similar permeability. Also like the depot membranes, the barrier membranes' permeability for larger molecules such as methylene blue is affected by the polymer network. Diaphragm cell studies indicate a methylene blue permeability across the barrier membranes of $3.57 \times 10^{-7} \text{ cm}^2/\text{s}$, only 10% of its permeability across a comparable amount of pure water.

3.3 *BMPR Devices*

Relying on depot swelling as the triggered action for solute release, it is critical that the depots be completely shrunken and hydrophobic initially, then become highly permeable to water and solute only after being triggered to release. The barrier, on the other hand, should be very hydrophilic, allowing water and solute to quickly diffuse across. This complicates integration of the two layers with a single sidewall material, as shown in Figure 10. Here the barrier and depot layers were cut to identical diameters (15 mm) and stacked in 5-pulse BMPR

Figure 10: Configurations and release profiles for BMPR devices. (Left) Configurations of two BMPR devices. Solute-free depots are placed on top to guarantee initial adhesion to the impermeable wall. Note that devices are not drawn to scale; actual aspect ratio resembles a coin. (Right) Solute release profiles for the devices at pH 7 (dotted) and pH 3 (solid). No release observed at pH 7, while regular discrete pulses are observed at pH 3.

devices. The devices had no discernable release during three days of immersion in pH 7 buffer.

At pH 3 we see sharp, discrete pulses at regular intervals corresponding to the barrier thickness.

The release time for each pulse is less than one hour, as predicted for depot layers only 100 μm

thick. The lag time between pulses, however, is only 2/3 as long as would be predicted for these

barriers based on Equation 3. Pictures of the device, recorded every 15 minutes, show that the

scavenger at the outer edge of each barrier layer is exhausted before the scavenger in the middle.

Moreover, in a few instances the depot swelled and delaminated before the scavenger in the

overlying barrier had even been consumed. Both problems point to poor contact between the

hydrated barrier layer and the wax sidewall, allowing acid to permeate around the edge of the

barrier and reach the depot layer earlier than planned. Despite the contact issues with these

devices, however, control over the pulse periodicity by altering the barrier thickness is still

conserved, with lag time between pulses increasing by a factor of four when the barrier thickness is doubled.

Barrier lag time can also be manipulated by altering the position of the scavenger within the barrier. As indicated in Equation 3, the lag time of the barrier varies linearly with the scavenger's center of mass: the effect of the scavenger is doubled when it is sequestered entirely at the downstream interface, while it is zero when all the scavenger is at the upstream interface and instantly consumed. Figure 11 shows devices constructed of identical films, with only the orientation of the barrier layers reversed. In these barriers, similar to the one shown via SEM in Figure 3C, the ZnO is distributed evenly across the first 42% of the film thickness, with the remainder being ZnO-free. These barrier layers were also cut 1 mm wider than the barriers used to demonstrate the contact issues in Figure 10, minimizing the opportunity for peripheral acid diffusion. When the ZnO-laden side of the barriers faces the device exterior, the lag time is only $5.5 (\pm 0.1)$ hrs, while when the orientation is reversed the lag time is $20.4 (\pm 0.2)$ hrs, nearly

Figure 11: Effect of scavenger position on release profiles for BMPR devices. (Left) Configurations of two BMPR devices made from the same films, but with the orientation of the barrier layers reversed. Scavenger in barrier layer is restricted to one side (shaded region) of layer. Note that devices are not drawn to scale; actual aspect ratio resembles a coin. (Right) Solute release profiles for the devices at pH 7 and pH 3. Lag time can be strongly manipulated by controlling the location of the scavenger in the barriers.

quadrupling the lag time without using any additional scavenger or increasing the barrier thickness. These lag times follow the relationship in Equation 3 within 3%.

One important feature of BMPR systems over single reservoir systems is the ability to deliver different chemicals at different times from the same device. Figure 12 shows a device with alternating depots of methylene blue and methyl orange. Positively-charged methylene blue releases from the depot very quickly and can show discrete pulses even at high loadings and short lag times, whereas methyl orange is negatively charged and shows bimodal release, with comparatively rapid release while the cationic depot amine groups are saturated with solute, followed by slower release as the remaining solute molecules are displaced by other anions. This requires a longer minimum lag time between discrete pulses of methyl orange, but has no impact on the other solutes delivered during the interim. In this case, each solute was released once per

Figure 12: Release of multiple solutes from a BMPR device. (Left) Configuration of a BMPR device containing doses of both methylene blue and methyl orange. Note that devices are not drawn to scale; actual aspect ratio resembles a coin. (Right) Solute release profiles for the devices at pH 7 and pH 3. Solutes are released sequentially in 12 hour intervals.

day, offset by 12 hours from each other, with lag times averaging 7% faster than the analytical predictions of Equation 3. Time-lapse videos of the release depicted in Figure 12 at pH 7 and at pH 3 are included as Supplementary Information.

In theory, there is no hard limit on how many pulses can be delivered by a single device, as underscored by Figure 13, which shows ten pulses from one device. In practice, however, one can see that as the number of layers increases, the opportunity for the contact problems mentioned above also increases, with pulses 3-6 delaminating 30% early. The rest of the pulses, and the last ones in particular, occurred within a few percent of their predicted time. A time-lapse video of the release depicted in Figure 13 is included as Supplementary Information.

4. Conclusions

Currently, pulsatile delivery of solute requires either labor-intensive manual application

Figure 13: How many pulses can one BMPR device provide? (Left) Configuration of a 10-pulse BMPR device. Note that devices are not drawn to scale; actual aspect ratio resembles a pair of coins. (Right) Solute release profiles for the devices at pH 7 and pH 3. Most solutes, including the last four, released at their analytically predicted time, but the opportunity for wall-barrier contact problems increases with the number of doses.

of every dose or expensive, complex devices. Barrier-mediated pulsatile release offers an elegant, materials-based alternative for pulsatile delivery. However, the linear dependence of erosion time on film thickness and the difficulty of maintaining perfect surface erosion across a wide range of thicknesses currently makes the use of polymer degradation as the timing mechanism for BMPR systems questionable. In this paper the BMPR approach has been decoupled from polymer degradation and instead linked to sacrificial scavenging of the external stimulant. This allows the delay time between pulses to be scaled across orders of magnitude with only modest changes in the barrier and opens the BMPR approach to a variety of external stimuli. PMMA/DMA hydrogels demonstrated complete immobilization of drug at pH 7 yet rapid release at pH 3, with characteristic swelling and release times that can be analytically predicted and manipulated. The acid stimulant for these depots can be blocked by ZnO-loaded polymer hydrogels, preventing solute release for a prescribed period of time, followed by rapid breakthrough of stimulant and release of solute. This delay time can be manipulated by the barrier thickness, its scavenger concentration and its scavenger location, with little hindrance of the stimulant afterwards. The number of pulses incorporated into a single device is limited only by the art of making them, with devices releasing up to 10 pulses demonstrated here. Importantly, different chemicals can be incorporated into each pulse, allowing automatic sequential delivery of multiple solutes.

This raises the spectre of inexpensive, customized delivery systems, where off-the-shelf layers are stacked in a particular configuration at the point of use and sealed into a simple polymer chip. There are no electronic or mechanical components, no power supply, and no circuitry, greatly reducing both the complexity and volume of the system, as well as its cost. The stimulant for the demonstrated system, citric acid, could be easily replaced by other organic

acids, potentially derived from other organic molecules enzymatically; gluconic acid derived from blood sugar being one example.

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