Time-Dependent Permeabilities of Hydrophobic, pH-Sensitive Hydrogels Exposed to pH Gradients

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Summary: pH-dependent changes in permeability to glucose of a poly(N-isopropylacrylamide-*co*-methacrylic acid) hydrogel membrane were measured with the membrane clamped between two cells. The donor cell was maintained at pH 7.0 and the receptor cell was subjected to a set of ramps in pH. Starting with the membrane in a state of high permeability to glucose, a ramp of decreasing pH led ultimately to shutoff of glucose flux. With passage of time at a fixed, low pH, however, permeability was restored to an intermediate value. Upon ramping pH back to its initial value, the initial permeability was restored. Based on light microscopy, the nonstationary permeability behavior is attributed to lateral stress-induced morphological changes that occur in a collapsed „skin" formed on the side of the membrane facing the acidic donor solution.

Keywords: hydrogels; hydrophobic; membranes; n-isopropylacrylamide; permeability; pH-sensitive; polyelectrolyte

Introduction

Hydrogels consisting of the lower critical solution temperature (LCST) monomer n-isopropyl acrylamide (NIPA) and acid comonomers are known to exhibit sharp, sometimes first-order volume phase transitions with change in environmental pH, temperature, or ionic strength.[1] Such transitions have been touted as the basis for drug delivery systems in which drug release rate is controlled by these variables, since partitioning and diffusion of drugs (or other molecules) into hydrogels is swelling-dependent.[2]

We have been investigating the feasibility of a drug delivery device that exhibits rhythmic changes in its permeability to peptide hormones such as gonadotropin releasing hormone (GnRH).[3-6] When this device is placed in an external medium of constant pH (7.0) and glucose concentration, rhythmic behavior is generated by a feedback instability between glucose permeation through a poly(n-isopropylacrylamide-*co*-methacrylic acid) [p(NIPA-*co*-MAA)] hydrogel membrane and enzymatic conversion inside the device of glucose to hydrogen ions, which modulate permeability of the membrane to glucose via a hysteretic

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swelling-collapse transition. The cyclic changes in membrane swelling that result from this process also lead to rhythmic switching of hormone permeability.

Investigations have shown that this system will only exhibit cyclic behavior if pH inside the device fluctuates sufficiently rapidly.[4-6] We have conjectured that slow changes in pH may drive the membrane into a stationary, intermediate glucose permeability state, perhaps due to stresses that build up in the hydrogel with time, these stresses arising from imposed pH gradients and from mechanical clamping of the membrane. This intermediate state leads to steady state behavior, where glucose flux into the device is exactly matched by hydrogen ion efflux. The present contribution summarizes evidence in favor of this view.

Experimental Methods and Results

Membrane Synthesis: 500 mg of a mixture containing 9:1 mol/mol NIPA (Kodak) and MAA (Polysciences) monomers, plus 0.5 mol% ethylene glycol dimethacrylate (EGDMA: Polysciences) was dissolved in 500 mg water and 500 mg methanol. This mixture was poured between two glass plates separated by a 250 μm spacer, and polymerization was initiated with 5 mg ammonium persulfate (Polysciences) and tetramethylenediamine (Aldrich), and carried out overnight at 10 °C. Hydrogel membranes were washed in methanol and water, and then conditioned in pH 4.5 saline solution at 37 °C.

Permeation Measurements: Membranes were mounted into the aperture (radius 1 cm) of a side-by-side diffusion cell (Crown Glass), with both magnetically stirred donor and receptor cells containing 80 ml saline solutions (50 mM NaCl). The cells were water-jacketed and maintained at 37 °C. The donor solution also contained 20 mM glucose, which was flowed through the donor cell at 5 ml/min. Programmed pH ramps in the donor cell were generated by an autotitrator (Brinkman) that delivered sufficient HCl to the donor compartment to set pH at time-dependent levels under control of a computer. (pH rise was made possible by inflow of essentially neutral saline glucose-solutions, but precise pH was still set by the autotitrator.) pH in the receptor compartment was fixed at 7.0 with a second autotitrator that delivered NaOH. In order to avoid using radiolabeled glucose to determine glucose flux across the membranes, an excess of glucose oxidase, catalase and gluconolactonase was added to the receptor phase. Appearance of glucose in the receptor phase led to rapid conversion of glucose to hydrogen ion, which was neutralized by NaOH from the autotitrator. Thus, glucose flux could be estimated from the rate of addition of NaOH to the receptor phase. Figure 1 illustrates the apparatus.

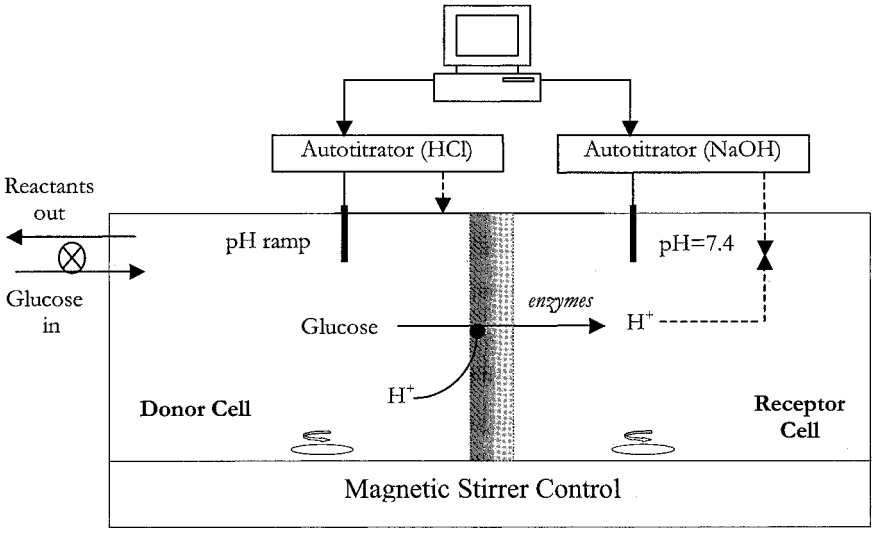

Figure 1. Illustration of apparatus to determine dynamic pH effect on membrane permeability to glucose. Glucose solution flows through the donor cell, whose pH varies in ramps under control of an autotitrator. Glucose permeating the membrane is enzymatically converted to H^+, which is neutralized by NaOH delivered from a second autotitrator. As pH in the donor cell is varied, H^+ from the donor cell affects swelling and hence permeability of the layer of hydrogel proximal to donor cell.

Figure 2 displays results for a particular pH-ramping program, which consisted of three identical sets of ramps. Each set begins with a downward ramp of donor cell pH from pH 4.8 to pH 4.3 over a 4-hour period, followed by a hold for 3 hours at pH 4.3, an upward ramp from pH 4.3 to 4.8 over 4 hours, and a hold at pH 4.8 for 1 hour. At the start of each set, the accumulated volume of NaOH titrated into the receptor cell rises steadily, indicating relatively high permeability of the membrane to glucose. As pH in the donor cell is progressively lowered, the volume of NaOH curve flattens out, indicating stoppage of glucose permeation. Eventually pH reaches its minimum value of 4.3, and after about an hour at that value, the volume of titrated NaOH curve starts to rise again, but at a rate that is not as high as the original rate. As pH is ramped back up to 4.8, the slope of the volume NaOH curve is restored to its original value.

Figure 2b shows the time derivative of the NaOH accumulation curve in Fig. 2a. This derivative provides an estimate of glucose permeation rate across the membrane. Despite the somewhat noisy appearance of the curve, which is due to the numerical differentiation (no smoothing operation was used), the curve demonstrates a consistent, repetitious

pattern of transition from high to essentially zero glucose permeability as pH is lowered below a threshold, followed by a later transition to an intermediate glucose permeability state while pH is held at 4.3. When pH is raised, the membrane restores its initial permeability to glucose. (During the "high permeability phase" there is some initial reduction in permeability as pH is lowered, due to progressive discharging of the MAA groups in the membrane, causing pre-transitional deswelling of the hydrogel.)

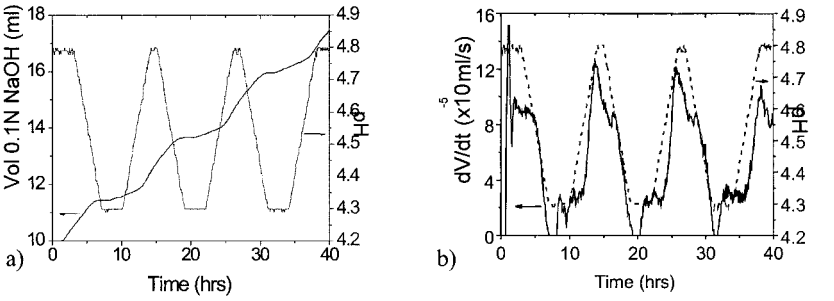

Figure 2. a) Cumulative volume of 0.1N NaOH added to neutralize H^+ ions generated enzymatically from glucose permeation across the membrane, with pH in receptor cell stat-ed at 7.0 and pH in the donor cell ramped up and down. b) Derivative (dV/dt) of cumulative NaOH volume-added curve, which reflects rate of glucose permeation across the membrane.

Observations of Membrane Morphology: As will be discussed below, the appearance of the intermediate permeability state is believed to be due to lateral stresses generated as the hydrogel progressively collapses. To investigate this possibility, the membrane was mounted in an apparatus that preserved the time-dependent cross-membrane pH difference, and that permitted visualization of changes in membrane morphology with time. The setup is illustrated in Figure 3. Cell A (analogous to the receptor cell in Fig. 1) was fixed at pH 7.0 by flow of 0.1N NaOH/50mM NaCl and pH in Cell B (analogous to the donor cell) was controlled by flow 0.01N HCl/50mM NaCl. Temperature in the cells was monitored by thermocouples, and was maintained at 37 °C by passing the acid and base solutions through coils in a water bath. Cell B was delimited by a metal ring, which also served as a means to bear down on and clamp the hydrogel membrane. The membrane was illuminated from below, and images were collected in a light microscope, with optics focused on the membrane surface adjacent to Cell B.

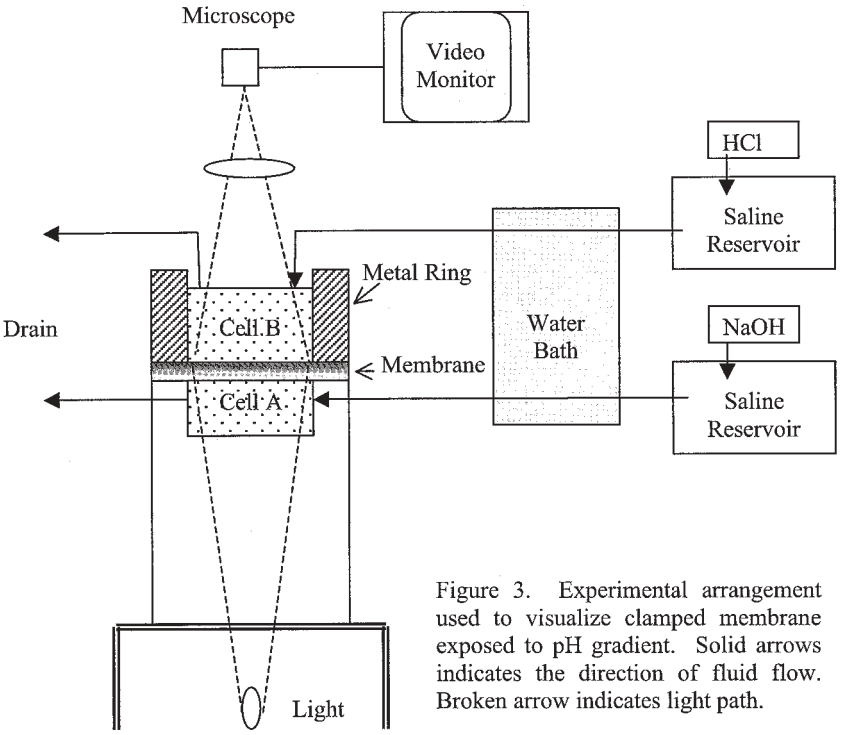

Figure 3. Experimental arrangement used to visualize clamped membrane exposed to pH gradient. Solid arrows indicates the direction of fluid flow. Broken arrow indicates light path.

Once mounted into the apparatus shown in Figure 3, the NIPA-*co*-MAA membrane was exposed to the pH program in Cell B shown in Figure 4. Starting at 4.9, pH in Cell B was switched to 4.25 at 2 hours, and switched back to 4.9 at 8.5 hours. Photomicrographic images of the membrane were collected at times denoted by P, Q, R, and S in Fig. 4. These images are shown in Figure 5.

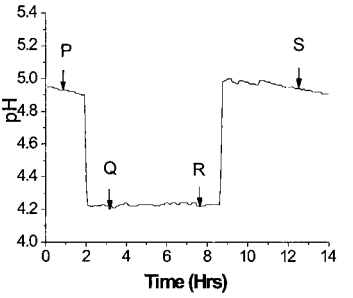

Figure 4. pH-time program in Cell B. Arrows label time points at which images of the gel were taken.

When exposed to relatively high pH (4.9) in Cell B (panel P), the membrane surface presents a uniform morphology, corresponding to the swollen state. Soon after pH is switched to 4.25 (panel Q), the surface develops coarse surface features due to collapse.

After holding pH at that lower value, the surface changes its morphology (panels R1 and R2), and the morphology is not uniform across the surface (compare R1 and R2). When pH is returned to 4.9, the membrane surface reverts to a morphology that is very similar to its initial form. When the membrane surface was exposed to pH 3 in Cell B, it adopted a tight, quasicellular morphology.

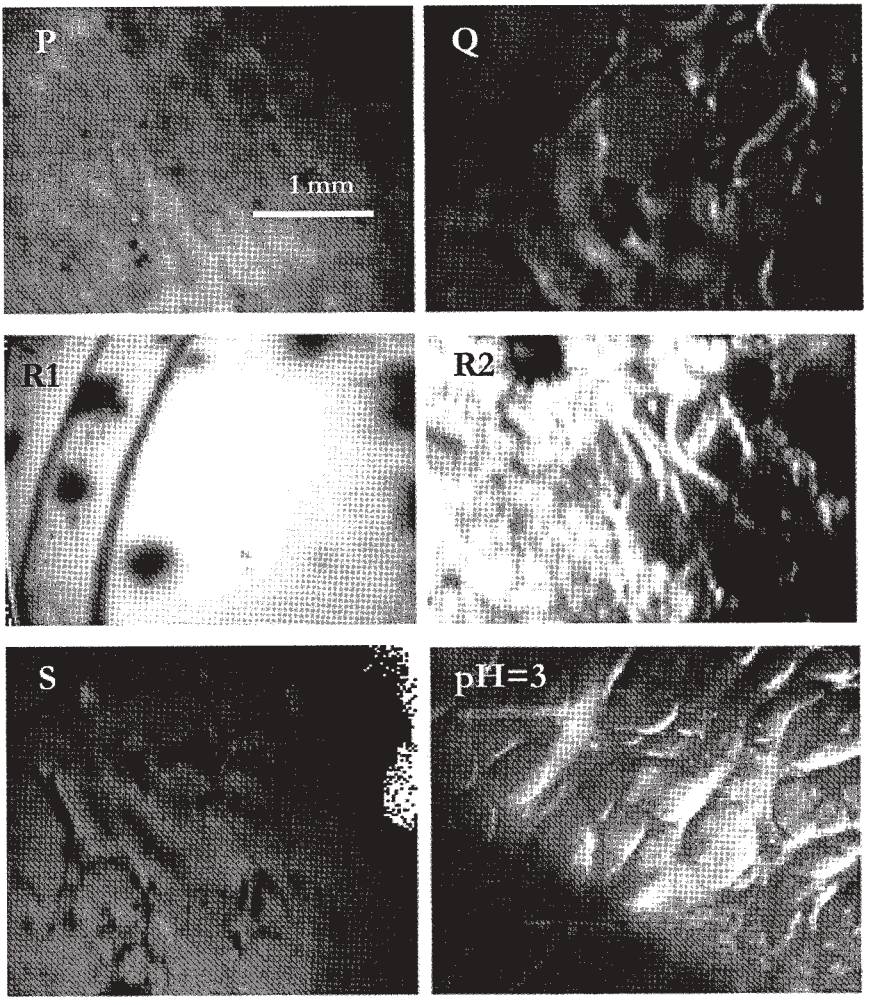

Figure 5. Micrographs of membrane surface facing Cell B taken at times indicated in Fig. 4. Panels R1 and R2 recorded near metal ring and near center of membrane, respectively. Bottom right panel is surface of membrane exposed to pH 3 in Cell B. Dark spots in R2 are due to air bubbles in the water below the membrane.

Discussion

Typically, volume phase transitions are measured in free-swelling circumstances, with all sides of the hydrogel exposed to the same media. Even under these relatively simple conditions, complicated transient phenomena such as surface patterns and cellular structures have been observed.[7-9] Such structures may persist indefinitely when mechanical constraints are placed on the gel, e.g. by bonding one side of the gel to a surface.[10,11] These structures reflect buckling instabilities that arise from mismatches between local swelling pressures and mechanical constraints during swelling, or „bubble" instabilities arising from „deswelling pressures" and low water permeabilities during deswelling. It has also been shown that isochorically constrained gels exhibit transitions in hydraulic permeability due to phase separation under environmental conditions that would promote collapse if free swelling were permitted.[12]

The present set of experiments is distinguished from previous work in two important ways. First, the hydrogel membranes in the present work were exposed to pH gradients, with one side of the gel always in contact with a medium at pH 7.4, while the other side was exposed to a medium at pH values that were ramped down and up between pH 4.8 and 4.3, which correspond to relatively swollen and collapsed states, respectively. The presence of the gradient, even if it is stationary, breaks the symmetry that is normally present when uniform conditions are applied, as in most experiments. Second, the hydrogel was clamped at its periphery, instead of being free or bonded to a substrate on one of its faces. In the latter case, deswelling may lead to buildup of internal pressures that cannot be relieved by water flow through a collapsed skin, and this leads to the appearance of bubbles on the surface. In the present system, the swollen hydrogel facing the pH 7.4 medium provided a „back door" for water escape during deswelling, and surface bubbles were not observed. (Such bubble formation has been observed in clamped hydrogel membranes in which both faces are exposed to low pH, deswelling conditions, however.[13])

Based on the considerations of the previous paragraphs, we propose a mechanism to explain the emergence of the intermediate glucose permeability state at low donor cell pH. The mechanism is illustrated in Figure 6. At „high" pH (say pH 4.8) in the donor cell of Fig. 1 (or Cell B of Fig. 2), the whole membrane is in a relatively swollen state (Fig. 6a), although there may be some gradation in swelling as one moves through the pH gradient inside the hydrogel. In this state, glucose permeates relatively freely through the

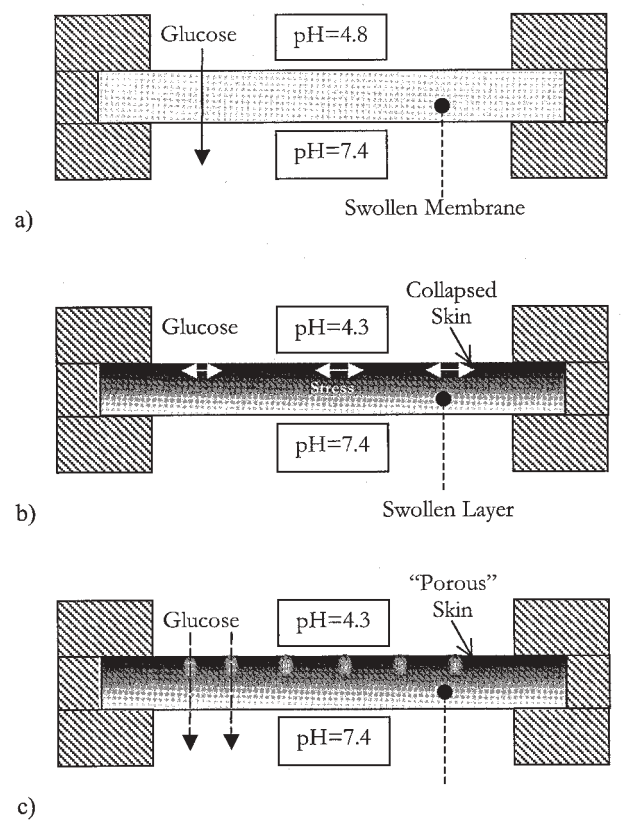

Figure 6. Schematic of changes in membrane permeability to glucose. Top of each panel corresponds to donor cell of Fig. 1, or Cell B of Fig. 2. a) Membrane in swollen, permeable state; b) Membrane with collapsed, impermeable skin, with development of lateral stress in skin due to clamping; c) Membrane with collapsed skin, in which „pores" have appeared due to phase separation in response to lateral stress, leading to intermediate glucose permeability.

membrane. When pH in the donor cell drops below a critical value, the MAA groups on the hydrogel proximal to the donor solution become sufficiently protonated to form a collapsed skin that is dense enough to block glucose permeation (Fig. 6b). In the absense of the lateral clamp, the skin would also pull inward, and the presence of the clamp therefore induces a lateral stress. Such stress might be mitigated immediately by separation of the skin into relatively swollen and condensed domains. However, the skin is attached to the swollen part of the membrane, which will oppose such separation. With passing time, however, the skin becomes thicker and stronger and the supporting swollen

layer becomes thinner and weaker, to the point where the the latter can no longer prevent phase separation of the former. Analogies to drying and cracking in mud flats and skinned fruit are apparent.

The repeatability of swelling cycles with cyclic pH stimulation, shown in Fig. 2, provides evidence, at least over the short term, that the permeable state (Fig. 6a) is restored when pH in the donor cell returns to its „high" (pH 4.8) value. Previous studies in which oscillations in membrane permeability were observed over a period of a week, also argue against the presence of short-term fatigue in these hydrogel membranes.

The presence of an intermediate permeability state of the membrane to glucose, attained after a certain time interval following collapse, may explain why the previously described rhythmic drug delivery device[5,6] ultimately settles down to stationary behavior. This device is based on unstable reciprocal feedback between glucose transport through the p(NIPA-*co*-MAA) membrane to the glucose oxidase-catalase-gluconolactonase enzyme system and the deswelling effect of hydrogen ions produced inside the device by these enzymes on the membrane's glucose permeability. When pH oscillations in the device are sufficiently rapid, the membrane flips between states of relatively high and near-vanishing permeability to glucose. When the pH oscillations are slow, however, the membrane has sufficient time to „find" the intermediate glucose permeability state, where glucose influx is balanced exactly by hydrogen ion efflux, and the membrane's charge profile, consistent with intermediate permeability, remains constant. pH and swelling oscillations in the device described in Refs. [5,6] are slowed down by the accumulation of gluconate, which is produced along with H^+ by the enzymatic reaction inside the device and acts as a pH buffer.

Conclusion

The present studies provide experimental support for the presence of an intermediate glucose permeability state that arises due to lateral stresses and ultimate domain formation in a rate-limiting skin layer that is formed proximal to the donor cell when pH in that cell is lowered. The transformation of the skin from a uniform, impermeable form to a fragmented, partially permeable form is believed to explain the ultimate cessation of oscillations in a membrane-enzyme feedback oscillator (rhythmic drug delivery device) that is observed when oscillator frequency decreases.

Despite the support provided by the present experiments, we regard these studies as preliminary. Experiments investigating the effect of pH slew rate are warranted, for

example. Perhaps more important, the light microscopy observations reported here, while suggestive, are crude. A integrated system in which glucose permeability and membrane morphology are tracked together is desirable. More quantitative tracking of membrane morphology with time might be provided by confocal microscopy, with fluorescent probes incorporated inside the hydrogel membrane. Such experiments are underway.

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