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Constant-Volume Hydrogel Osmometer: A New Device Concept for Miniature Biosensors

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A new type of biosensor is proposed that combines the recognition properties of "intelligent" hydrogels with the sensitivity and reliability of microfabricated pressure transducers. In the proposed device, analyte-induced changes in the osmotic swelling pressure of an environmentally responsive hydrogel are measured by confining it within a small implantable enclosure between a rigid semipermeable membrane and the diaphragm of a miniature pressure transducer. Proof-of-principle tests of this device were performed in vitro using pH-sensitive hydrogels, with osmotic deswelling data for the same hydrogels used as a benchmark for comparison. The swelling pressure of the hydrogel was accurately determined from osmotic deswelling measurements against reservoirs of known osmotic stress. Values of swelling pressure vs salt concentration measured with a preliminary version of the sensor agree well with osmotic deswelling results. Through modification of the hydrogel with various enzymes or pendant binding moieties, the sensor has the potential to detect a wide range of biological analytes with good specificity.

Introduction

Clark and Lyons pioneered the development of biosensors in 1962, using an amperometric technique in which an enzymatic reaction involving the analyte produced a current in an electrode.[1] Sensor development continues to be an extremely active research area, due to the immense practical value of sensors in fields ranging from health care to environmental monitoring to the agricultural and chemical industries.[2−5] For example, due to great medical need, there is a major effort underway to develop a painless and inexpensive glucose sensor for continuous monitoring of blood glucose levels in diabetic patients.[6] The goal of any sensor design is the accurate and quantitative determination of the concentration of an analyte by detecting physical and/or chemical signals proportional to the analyte concentration.[5] The transducer may be electrochemical, piezoelectric, thermoelectric, acoustic, or optical in nature, depending on the analyte property being measured. We present here proof-of-principle results for a novel approach that takes advantage of recent advances in microfabricated pressure transducers[7] and "intelligent" polymer hydrogels.[8] The basic principle of operation is sketched in Figure 1.

Figure 1A shows a conventional approach for use of *stimuli-responsive hydrogels*. A stimulus-reponsive hydrogel is a cross-linked polymer network that changes its swelling ratio in response to some stimulus in the environment such as pH, temperature or concentration of a particular analyte.[9] A *glucose-sensitive hydrogel* changes its swelling ratio in response to the environmental glucose concentration. In one

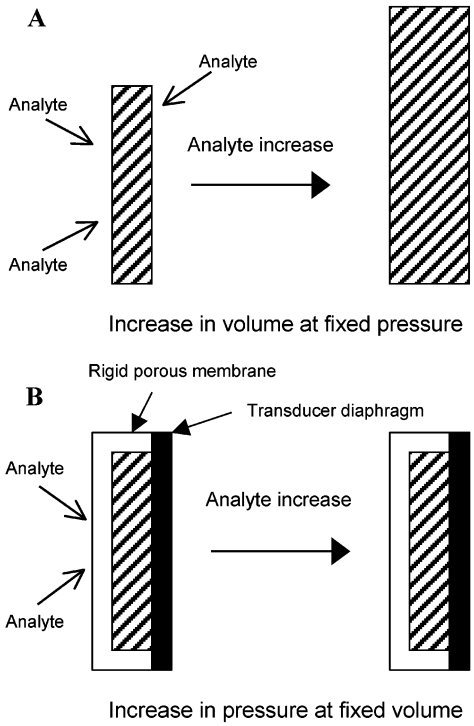

Figure 1. Schematic representation of new sensor approach: (A) swelling of unconfined responsive hydrogel; (B) responsive hydrogel at fixed volume in sensor between rigid porous membrane and diaphragm of miniature pressure transducer.

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of several possible approaches,[10] the backbone of the glucose-sensitive hydrogel contains pendant phenylboronic acid (PBA) moieties that reversibly bind to glucose. When glucose complexes to PBA, this lowers the apparent pK_a value of the boronic acid group, and the fraction of charged borate anions in the hydrogel is increased.[10] In Figure 1A, an increase in the environmental concentration of an analyte such as glucose causes an increase in the local glucose concentration within the hydrogel. If the glucose-sensitive hydrogel, contains PBA moieties, then the increase in local glucose concentration increases the fraction of charged borate anions, which in turn produces a temporary decrease in the chemical potential of water within the hydrogel. Hence the *unconfined* hydrogel absorbs water and swells, and the swelling continues until the favorable free energy of mixing is balanced by the unfavorable stretching of hydrogel polymer chains.[11] If a film made from this hydrogel encloses a drug reservoir, then the increase in permeability due to swelling may be sufficient to release the drug into the environment.[12−14] However, response times for hydrogel swelling or shrinking are notoriously long, because the kinetics are often controlled by polymer network motion through the solvent, and the collective diffusion coefficient that governs the process is much smaller than the translational diffusion coefficient of small molecule analytes.[9] The problem of slow response time can be mitigated by using a constant volume approach that we favor for the use of hydrogels in biosensors, as shown schematically in Figure 1B. In Figure 1B, the same hydrogel is confined to a volume that is essentially constant, between a rigid porous membrane and a low compliance pressure transducer. Now when the analyte concentration increases in the gel, water will diffuse into the hydrogel until the favorable free energy of mixing is balanced by the increase in mechanical pressure within the enclosure. Changes in analyte concentration can be detected from measurements by the pressure transducer, and the device is essentially a miniature osmometer. Because of the fixed volume, kinetics should be limited by the diffusion of the analyte, not by the diffusion of the polymer network through the solvent. In addition, it may be possible to use very thin hydrogel slices, since the osmotic pressure is independent of hydrogel thickness. These factors may lead to an unusually fast response time for an implantable hydrogel-based sensor.

The sensor should be very versatile, since by modifying the hydrogel one will be able to tailor the sensor to respond to a wide variety of analytes, provided that the analyte does not bind irreversibly to the gel matrix. For example, for specific response to glucose, one can physically immobilize the enzyme glucose oxidase (GOX) within the matrix of a pH-sensitive hydrogel.[13−15] Alternatively, to obtain a non-enzymatic sensor, one can chemically immobilize glucose-binding moieties into the gel such as concanavalin A[16,17] or phenylboronic acid.[10] The competitive binding/reversible cross-linking approach of Miyata and co-workers[18] can be used to prepare hydrogels with specific response to any analyte for which antibodies can be prepared. If necessary, a reference hydrogel without analyte-specific interactions can also be implanted and used to remove nonspecific response of the sensor by subtraction.

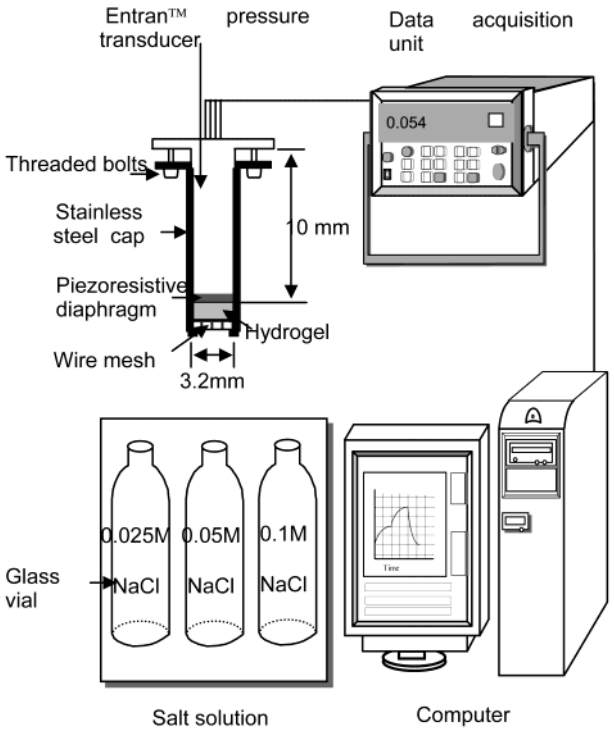

Figure 2. Preliminary version of sensor and experimental setup for proof-of-principle tests. The hydrogel is confined between a rigid wire mesh and an Entran piezoresistive pressure transducer with a stainless steel pressure-sensing area. The pressure transducer was calibrated in house up to 345 kPa.

Although the implantable osmometer/biosensor is still in an early phase of development, we have tested the basic concept using a preliminary version of the device that is sketched in Figure 2. The results of these in vitro experiments will be presented along with new characterization results for the hydrogels used in the device. The concept of making pressure measurements in order to obtain hydrogel osmotic properties goes back at least 30 years to van de Kraats.[19] However, the concept of using hydrogels and pressure transducers to detect analytes in the environment appears to be novel.

Thermodynamic Definition of Swelling Pressure

Unconfined hydrogels swell until the total change in free energy, ΔF_{tot}, reaches a minimum or, equivalently, until the chemical potential of each mobile species becomes equal in the coexisting phases.[11] For a nonionic hydrogel, the mixing of water with the polymer makes a negative contribution to the free energy (ΔF_{mix}), while the stretching of the hydrogel network makes a positive contribution (ΔF_{el}). For a polyelectrolyte hydrogel, there is an additional negative contribution associated with the mixing of water with the counterions (ΔF_{ion}). Assuming these terms are independent, we can write[11,20,21]

$$\Delta F_{tot} = \Delta F_{mix} + \Delta F_{el} + \Delta F_{ion} \quad (1)$$

In an osmotic swelling experiment the measurable quantities involve derivatives of the free energy,[11] i.e.

$$\Pi_{tot} = -(\partial \Delta F_{tot}/\partial n_1)/V_1 = \Pi_{mix} + \Pi_{el} + \Pi_{ion} \quad (2)$$

where Π_{tot} is the swelling pressure of the gel, Π_{mix}, Π_{el}, and Π_{ion} are the mixing, elastic, and ionic contributions of Π_{tot}, respectively, V_1 is the molar volume of water, and n_1 is the number of moles of water. At swelling equilibrium for an unconfined hydrogel, Π_{tot} must equal zero; hence, eq 2 can be used to predict equilibrium swelling ratios. For a confined hydrogel (Figure 1B), one can compensate for a nonzero value of the total swelling pressure by applying a hydrostatic pressure difference between the gel and the surrounding solution.[22] For similar hydrogels as studied here, Siegel attempted to predict the unconfined swelling behavior with Π_{el} calculated using rubber elasticity theory, Π_{mix} calculated using the Flory−Huggins model, and Π_{ion} calculated using classical Donnan equilibrium theory.[23] The resulting predictions were only qualitatively successful because of specific ion effects.[23]

Experimental Methods

Gel Preparation. Proof-of-principle tests of our sensor were performed using pH-sensitive hydrogels synthesized by free radical cross-linking copolymerization of hydroxypropyl methacrylate (HPMA, Polysciences, Inc.), (*N*,*N*-dimethylamino)ethyl methacrylate (DMA, Polysciences, Inc.) and cross-linker tetraethylene glycol dimethacrylate (TEGDMA, Polysciences, Inc.) at the nominal mole ratio 70:30:02, respectively. Details of the synthesis are discussed in ref 15, where it is also shown that the hydrogels swell at pH values below about 7 and that the pH response is largely unaffected by the presence in the gel of enzymes glucose oxidase and catalase. Immobilization of glucose oxidase in a pH-sensitive hydrogel produces a glucose-sensitive hydrogel, because the enzyme converts glucose to gluconic acid and lower the pH value within the gel.[13−15,24] However, no enzymes were immobilized in the pH-sensitive hydrogels studied here. Gelation was performed in a glass mold containing a disk-shaped cavity of thickness 0.4, 0.8, or 3.0 mm. Hydrogels with the larger thickness were used in the osmotic deswelling experiments, whereas hydrogels prepared from the thinnest mold were used in the prototype sensor.

Swelling Measurements (Unconfined Hydrogels). Aqueous solutions at various ionic strengths were prepared using deionized water and NaCl (Sigma). A given hydrogel sample was immersed at room temperature in an aqueous solution that was replaced frequently during the swelling process. Periodically, the gel sample was withdrawn from the solution and weighed after removal of excess surface solution by light blotting with a laboratory tissue. The swelling ratio Q for a gel sample was calculated at W_w/W_d, where W_w and W_d are swollen and dry weights, respectively. The swelling ratio so defined is the inverse of the polymer weight fraction. Dry weights were determined by weighing gel samples after washing in deionized water and drying for at least 5 days at 60 °C. Swelling ratios were measured for three to four small samples taken from the same reaction mold, and the standard deviation was taken as an estimate of the uncertainty in the measured swelling ratio.

Deswelling Measurements. Deswelling of the hydrogel samples was achieved by enclosing them in a dialysis bag surrounded by a large reservoir of an aqueous solution of poly(vinylpyrrolidone) (PVP, 29 kDa) of known osmotic pressure.[25,26] The dialysis bag is permeable to salt but impermeable to polymer. At equilibrium, the swelling pressure of the hydrogel inside the dialysis bag is equal to the osmotic pressure exerted by the solution outside. At this point, gel samples were removed from the dialysis bag, weighed, and dried. This procedure gives for each hydrogel sample the dependence of swelling pressure Π_{tot} on swelling ratio Q.

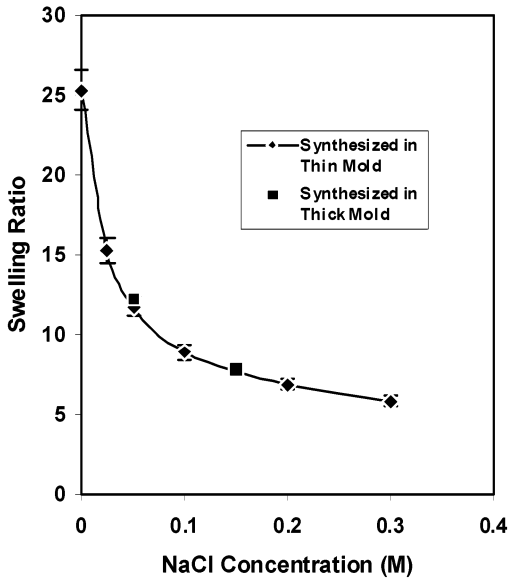

Figure 3. Equilibrium swelling ratio for unconfined hydrogel as a function of environmental salt concentration (pH value ≈ 6), as measured on samples synthesized in thick mold (3.0 mm) and in thin mold (0.8 mm).

Swelling Pressure Measurements in Sensor. The preliminary version of the sensor (Figure 2) was built using an off-the-shelf piezoresistive pressure transducer from Entran, Inc. (model EPB-501). Hydrogel samples synthesized using the thinner glass mold (0.4 mm thickness) were washed and stored in PBS buffer (pH = 7.4) for at least 48 h before use. A stainless steel punch was used to cut gel samples of appropriate diameter for insertion into the sensor. In the sensor, a stainless steel cap with threaded bolts confines the hydrogel sample between a rigid wire cloth mesh (80 mesh) through which salt can pass and the stainless steel sensing area of the pressure transducer. For each hydrogel sample, to ensure good physical contact, the threaded bolts were tightened until the signal from the pressure transducer reached 5 mV (15.6 kPa) before any changes in swelling pressure were measured.

Results

In Figure 3, the equilibrium swelling ratio Q_{eq} of the unconfined hydrogel is plotted against the salt concentration of the surrounding solution at a fixed pH value of about 6 (i.e., the pH value of air-saturated deionized water[27]). Since this pH value is below the transition value (≈7.4) of this basic pH-sensitive hydrogel,[15] almost all of the tertiary amine groups pendant to the comonomer DMA should be positively charged. The ionic contribution to the swelling pressure Π_{ion} can be estimated by the Donnan theory, which predicts that this term is approximately proportional to the difference in mobile ion concentration between the hydrogel and the surrounding solution.[11] The highest swelling ratio is observed in pure water. In this case, there are no salt ions in the surrounding solution, but a high concentration of mobile counterions must be present inside the gel in accordance with the electroneutrality requirement. When salt is added to the solution, this reduces the difference between ion concentrations inside and outside the gel, though a portion of the added salt will diffuse into the network. Consequently the equilibrium swelling ratio gradually decreases with increasing salt concentration in Figure 3.

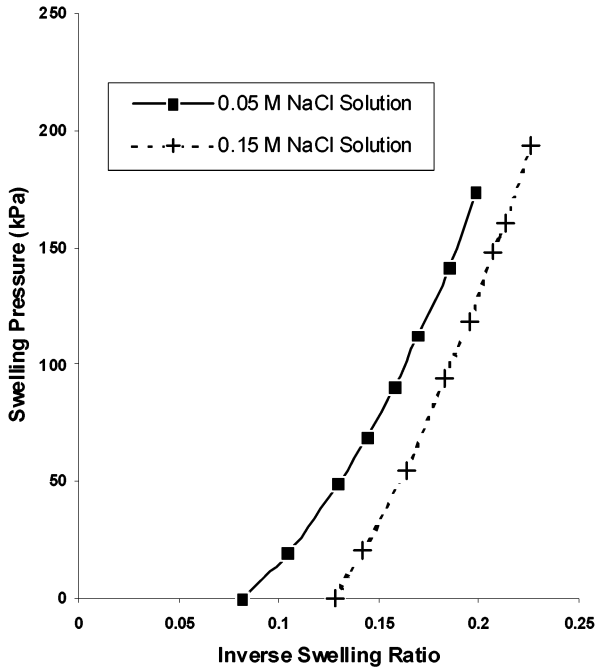

Figure 4. Swelling pressure of hydrogel vs inverse of the swelling ratio as obtained from osmotic deswelling experiments at pH ≈ 6 and two different values of the environmental salt concentration.

Results are shown for two different values of sample thickness. Evidently the hydrogel thickness has little effect on the equilibrium swelling ratio. At each value of the salt concentration in Figure 3, the hydrogel adopts that value of the swelling ratio which gives zero swelling pressure ($\Pi_{tot} = 0$). However, by placing the hydrogel on one side of a dialysis membrane, and by placing an osmotic stressing agent on the other side of the membrane, we can force the hydrogel to adopt a smaller swelling ratio at which the swelling pressure Π_{tot} is greater than zero. The results of these osmotic deswelling experiments are shown in Figure 4. In Figure 4, the swelling pressure is plotted against the inverse of the nonequilibrium swelling ratio, at two different fixed values of the salt concentration (0.05 and 0.15 M) in deionized water. The salt can pass through the dialysis membrane whereas the osmotic stressing agent (PVP) cannot.

Both curves in Figure 4 are well fit by the scaling expression[28]

$$\Pi_{tot} = A[Q^{-n} - (Q_{eq})^{(1/3)-n}(Q^{-1/3})] \qquad (3)$$

where Q is the swelling ratio, Q_{eq} is the equilibrium swelling ratio at the same salt concentration, n is the excluded volume exponent which depends on the thermodynamic quality of the solvent, and A is a constant characteristic of the particular polymer−solvent system. The best fit values of A are 7695 kPa at 0.05 M NaCl and 7350 kPa at 0.15 M NaCl. The best fit values of the excluded volume exponent are 2.22 at 0.05 M NaCl and 2.15 at 0.15 M NaCl. In this work, eq 3 is used as an empirical expression for fitting the data, but it should be pointed out that the equation was originally derived for neutral gels; hence, it is surprising that it works so well here. We are currently performing additional experiments in order to determine why this should be the case. For proof-of-principle tests, thin samples (0.4 mm) of the same hydrogel were placed into the sensor sketched in Figure 2.

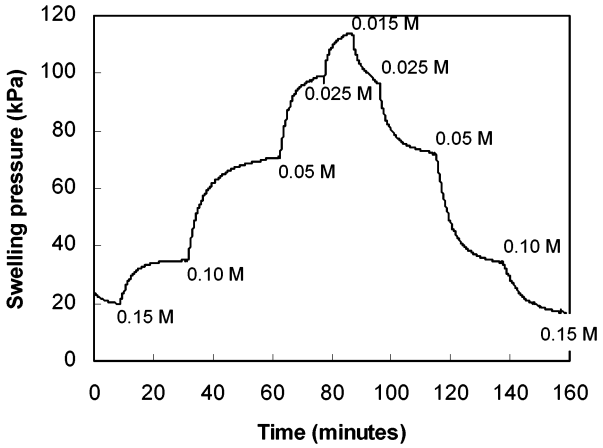

Figure 5. Swelling pressure vs time for constant-volume hydrogel in prototype sensor transferred between various salt solutions at fixed pH. Initially the sensor was equilibrated with a solution of concentration 0.15 NaCl, and the sensor was suddenly transferred at various times between solutions of differing salt concentration. The salt concentration of the surrounding solution at various times is given in the figure.

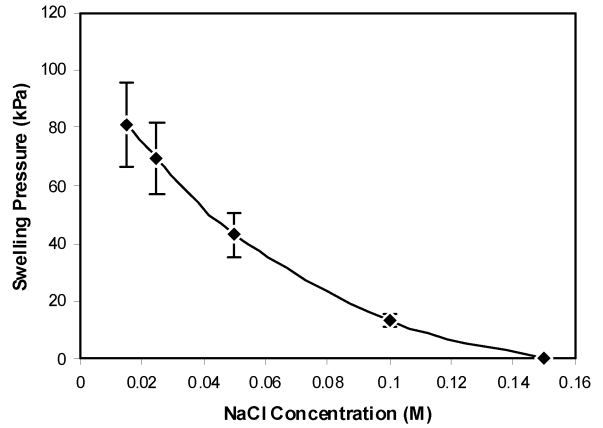

Figure 6. Change in swelling pressure vs external salt concentration at fixed pH for constant-volume hydrogel in prototype sensor. The measured swelling pressure at 0.15 M NaCl is used as the reference value.

Figure 5 shows the change in the value of the swelling pressure measured by the piezoresistive transducer for a cyclic process in which the sensor is placed successively into different aqueous solutions with identical pH values (≈6) but with slightly different salt concentrations. Evidently, the change in swelling pressure is easily detected for each step, and the overall cyclical process shows little or no hysteresis. All of the results shown in Figure 5 were obtained using hydrogel samples taken from the same reaction mold, and reproducibility is excellent. The swelling pressure measured with the preliminary version of the sensor is responsive to external salt concentration over a wide range, as shown by the results in Figure 6. The error bars in Figure 6 are primarily due to variations in hydrogels from different synthesis batches.

In Figure 4, the vertical distance between the two curves is almost constant at a value of 45−47 kPa. This means that when the external salt concentration is reduced from 0.15 to 0.05 M, it takes about 45−47 kPa of additional *osmotic stress* to keep the hydrogel volume fixed. If our prototype sensor is accurate, it should take about 45−47 kPa of additional

mechanical pressure to keep the hydrogel volume fixed for the same change in external salt concentration. From Figure 6, the value measured with the sensor is 43 ± 8 kPa, including hydrogel batch-to-batch variations. We consider this to be acceptable agreement for the preliminary version of the sensor.

Discussion and Conclusions

We have shown that changes in the osmotic pressure of a thin slice (0.4 mm) of a responsive hydrogel can be accurately measured by confining it to a small rigid container with a rigid porous membrane (i.e., a wire mesh) and a miniature pressure transducer. Osmotic pressure values for the thin hydrogel slices measured with the novel device agree well with results of classical osmotic deswelling experiments performed on much larger hydrogel samples. These results demonstrate the feasibility of a new sensor concept that can be applied to many different analytes, but in this paper, we have not applied the concept to a particular biosensor. The particular hydrogel studied in this paper could be used for glucose detection, but we do not believe that its chemical structure is optimum for this purpose. Currently we are investigating issues such as biocompatibility, response time and sensitivity when the sensor concept is applied to glucose detection. We propose to apply the sensing concept of Figure 1B to the field of continuous glucose monitoring as follows. We envision a device that is a small wireless capsule, capped at one end by a rigid semipermeable membrane that is permeable to small molecules but impermeable to blood clots, cells, and biomacromolecules. The interior of the capsule will contain a glucose-sensitive hydrogel confined to a fixed volume between the semipermeable membrane and a miniature pressure transducer. We envision implanting the device in the subcutaneous layer of the skin in order to continuously monitor the concentration of glucose within the patient's interstitial fluid.[29] The analyte will diffuse into the capsule, thereby changing the swelling pressure of the hydrogel, and the pressure signal will be transmitted wirelessly by radio waves to a receiver located outside the body. The envisioned device can also be powered using radio waves from a remote source.[30] Research on microelectromechanical systems (MEMS) technology[31] has led to remarkable advances in ultraminiature implantable pressure transducers suitable for applications such as monitoring intraocular pressure.[30,32] Using a similar pressure transducer, the implantable biosensor that we envision might have a diameter as small as 250 μm and a length of 1−5 mm. Possible advantages of the proposed device are substantial. The proposed sensor should have a simple and robust design, with a clear scientific mechanism that relates the sensor signal to the glucose concentration. Glucose-sensitive hydrogels based on phenyboronic acid moieties are highly specific to glucose, and independent of physiological oxygen levels.[10] According to our preliminary experimental results, sensor response time can be reduced substantially by using thinner porous hydrogel slices or hydrogel beads compacted into a thin layer.

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References and Notes

(1) Clark, L. C., Jr.; Lyons, C. *Ann. N.Y. Acad. Sci.* **1962**, *148*, 133.
(2) Yager, P. In *Biomaterials Science: An Introduction to Materials in Medicine*; Hoffman, A. S., Schoen, F. J., Lenons, J. E., Eds.; Academic Press: San Diego, CA, 1996; pp 375−388.
(3) Leung, J. H. T.; Bouvrette, P.; Male, K. B. *TIBTECH* **1988**, *6*, 369.
(4) Wilkins, E. S.; Atansov, P. *Med. Eng. Phys.* **1996**, *18*, 273.
(5) Ellis, A. B., Walt, D. R.; Eds. *Chem. Rev.* **2000**, *100*, 2477.
(6) Report "Diabetes: Innovation and Growth" by Medical Data International, Englewood, CO; June/July 1998, listed on their website at www.medicaldata.com/mpm/98HighLights/6-7-98/6-7-98-2.asp.
(7) Tandeske, D. *Pressure Sensors*; Marcel Dekker: New York, 1991.
(8) Galaev, I. Y.; Mattiasson, B. *Trends Biotechnol.* **1999**, *17*, 335.
(9) Gehrke, S. H. *Adv. Polym. Sci.* **1993**, *110*, 81.
(10) Kataoka, K.; Miyazaki, H.; Okano, T.; Sakurai, Y. *Macromolecules* **1994**, *27*, 1061.
(11) Flory, P. J. *Principles of Polymer Chemistry*; Cornell University Press: Ithaca, NY, 1953.
(12) Ishihara, K.; Kobayashi, M.; Shionohara, I. *Makromol. Chem. Rapid Commun.* **1983**, *4*, 327.
(13) Kost, J.; Horbett, T. A.; Ratner, B. D.; Singh, M. J. *Biomed. Mater. Res.* **1984**, *19*, 1117.
(14) Albin, G. W.; Horbett, T. A.; Miller, S. R.; Ricker, N. L. *J. Controlled Release* **1987**, *6*, 267.
(15) Jung, D.-Y.; Magda, J. J.; Han, I. S. *Macromolecules* **2000**, *33*, 3332.
(16) Jeong, S. Y.; Kim, S. W.; Eenink, M. J. D.; Feijen, J. *J. Controlled Release* **1984**, *1*, 57.
(17) Miyata, T.; Jikihara, A.; Nakamae, K.; Hoffman, A. S. *Macromol. Chem. Phys.* **1996**, *197*, 1135.
(18) Miyata, T.; Asami, N.; Uragami, T. *Nature (London)* **1999**, *399*, 766.
(19) Van de Kraats, E. J. *Recl. Trav. Chim. Pays-Bas* **1968**, *87*, 1137.
(20) Dusek, K.; Prins, W. *Adv. Polym. Sci.* **1969**, *6*, 1.
(21) Horkay, F.; Tasaki, I.; Basser, P. J. *Biomacromolecules* **2000**, *1*, 84.
(22) Everett, D. H. *Basic Principles of Colloid Science*; Royal Society of Chemistry: London, U.K., 1988; pp 82−89.
(23) Siegel, R. A. *Adv. Polym. Sci.* **1993**, *109*, 233.
(24) Siegel, R. A.; Firestone, B. A. *Macromolecules* **1988**, *21*, 3254.
(25) Vink, H. *Europ. Polym. J.* **1971**, *7*, 1411.
(26) Horkay, F.; Zrinyi, M. *Macromolecules* **1982**, *15*, 815.
(27) Ebbing, D. D. *General Chemistry*, 4th ed.; Houghton Mifflin: Palo Alto, CA, 1993; p 678.
(28) Horkay, F.; Hecht, A. M.; Geissler, E. *J. Chem. Phys.* **1989**, *91*, 2706.
(29) Roe, J. N.; Smoller, B. R. *Crit. Rev. Ther. Drug Carrier Syst.* **1998**, *15*, 199.
(30) Walter, P.; Schnakenberg, U.; vom Bogel, G.; Kruger, C.; Dinslage, S.; Ludtke Handjery, H. C.; Richter, H.; Mokwa, W.; Diestelhorst, M.; Krieglstein, G. K. *Ophthalmic Res.* **2000**, *32*, 278.
(31) Bishop, D.; Gammel, P.; Giles, C. R. *Phys. Today* **2001**, *Oct.*, 38−44.
(32) Ultraminiature pressure sensor and method of making same. US Patent 4,881,410, 1989.

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