Anal. Chem. **2009,** *81,* 3630–3636

Determination of Glucose Levels Using a Functionalized Hydrogel−Optical Fiber Biosensor: Toward Continuous Monitoring of Blood Glucose in Vivo

Sven Tierney,[†] Berit M. Hasle Falch,[‡] Dag Roar Hjelme,[‡] and Bjørn Torger Stokke*,[†]

Biophysics and Medical Technology, Department of Physics, The Norwegian University of Science and Technology, NTNU, NO-7491 Trondheim, Norway, and Invivosense ASA, Leangenvegen 7, NO-7044, Trondheim, Norway

Glucose-selective optical sensors were fabricated by incorporating 3-phenylboronic acid and a tertiary amine, dimethylaminopropylacrylamide, into a hydrogel matrix. Determination of glucose in solution is based on the glucose-induced contraction of the hydrogel. The gel was fabricated on the end of an optical fiber, and the optical length was measured by an interferometric technique. Previously it was found the gel could be tuned for enhanced glucose sensitivity and selectivity by varying the 3-phenylboronic acid/tertiary amine ratio. The properties of the responsive hydrogel as a glucose sensor were determined in more detail with respect to swelling kinetics and equilibrium swelling degree. Temperature effects, size variation, molecular interference, and reversibility were addressed. Results showed there was a good degree of reversibility, both for equilibrium swelling and swelling kinetics. Fabricated hydrogel sensors with slight differences in size yielded an overlapping relative response indicating an excellent degree of sensor reproducibility. The sensor proved to be temperature-dependent; by increasing the temperature from 25 to 37 °C, the swelling was about 4-fold more rapid, and a concomitant decrease in equilibrium swelling was seen. Identified interference from other analytes with determination of glucose was used a basis for selecting ethylenediaminetetraacetic acid (EDTA) as an anticoagulant for in vitro determination of glucose concentration in blood plasma. Glucose measurements performed in blood plasma were promising, showing that the sensor is capable of measuring physiological glucose levels in blood with a minimal effect from interfering molecules. The obtained results indicate that the developed sensor is a candidate for continuous monitoring of glucose in blood.

Determination of glucose under physiological conditions is an ongoing intensive research area due to the large number of humans with diabetes, estimated to be 177 million in the year 2000 by the World Health Organization. There are already a variety of instruments available for glucose monitoring, most of them exclusively based on intermittent testing. For example, current monitoring of glucose levels for diabetics requires the person to prick his or her fingers for blood sampling several times a day. On the other hand, there are few instruments available for continuous glucose measurements.[1] In the context of diabetes, a reliable system for continuous glucose monitoring would be imperative for the realization of a self-regulating insulin system. In addition, there is a need for continuous glucose monitoring of critically ill patients. This is due to that hyperglycemia has been associated with risk of mortality and morbidity in the intensive care unit.[2] The majority of conventional glucose sensors rely on an enzymatically based sensing scheme, mainly glucose oxidase[3,4] and in some cases glucose dehydrogenase.[5] Detection has been achieved both electrochemically and also colorimetrically/photometrically. An alternative approach has been to use a synthetic recognition agent, phenylboronic acid.[6] Primarily used in spectroscopic techniques for carbohydrate detection,[7−9] this receptor has also proven to be applicable when integrated in responsive hydrogels.[10−12] Over the past 5−8 years intensive investigation on hydrogel-based sensors has been carried out originally by the group of Asher[10,13,14] applying a crystalline colloidal readout

* To whom correspondence should be addressed. E-mail: bjorn.stokke@ntnu.no.

[†] The Norwegian University of Science and Technology.

[‡] Invivosense ASA.

(1) Kondepati, V. R.; Heise, H. M. *Anal. Bioanal. Chem.* **2007**, *388*, 545–563.
(2) Williams, L. S.; Rotich, J.; Qi, R.; Fineberg, N.; Espay, A.; Bruno, A.; Fineberg, S. E.; Tierney, W. R. *Neurology* **2002**, *59*, 67–71.
(3) Malitesta, C.; Palmisano, F.; Torsi, L.; Zambonin, P. G. *Anal. Chem.* **1990**, *62*, 2735–2740.
(4) Hale, P. D.; Boguslavsky, L. I.; Inagaki, T.; Karan, H. I.; Lee, H. S.; Skotheim, T. A.; Okamoto, Y. *Anal. Chem.* **1991**, *63*, 677–682.
(5) Zhang, M. G.; Smith, A.; Gorski, W. *Anal. Chem.* **2004**, *76*, 5045–5050.
(6) James, T. D.; Shinkai, S. In *Host−Guest Chemistry*; Penadés, S., Ed.; Springer-Verlag Berlin: Berlin, Germany, 2002; Vol. 218, pp 159−200.
(7) Eggert, H.; Frederiksen, J.; Morin, C.; Norrild, J. C. *J. Org. Chem.* **1999**, *64*, 3846–3852.
(8) James, T. D. In *Creative Chemical Sensor Systems*; Schrader, T., Ed.; Springer-Verlag Berlin: Berlin, Germany, 2007; Vol. 277, pp 107−152.
(9) Gamsey, S.; Suri, J. T.; Wessling, R. A.; Singaram, B. *Langmuir* **2006**, *22*, 9067–9074.
(10) Asher, S. A.; Alexeev, V. L.; Goponenko, A. V.; Sharma, A. C.; Lednev, I. K.; Wilcox, C. S.; Finegold, D. N. *J. Am. Chem. Soc.* **2003**, *125*, 3322–3329.
(11) Kabilan, S.; Marshall, A. J.; Sartain, F. K.; Lee, M. C.; Hussain, A.; Yang, X. P.; Blyth, J.; Karangu, N.; James, K.; Zeng, J.; Smith, D.; Domschke, A.; Lowe, C. R. *Biosens. Bioelectron.* **2005**, *20*, 1602–1610.
(12) Lee, Y. J.; Pruzinsky, S. A.; Braun, P. V. *Langmuir* **2004**, *20*, 3096–3106.
(13) Ben-Moshe, M.; Alexeev, V. L.; Asher, S. A. *Anal. Chem.* **2006**, *78*, 5149–5157.
(14) Alexeev, V. L.; Das, S.; Finegold, D. N.; Asher, S. A. *Clin. Chem.* **2004**, *50*, 2353–2360.

10.1021/ac900019k CCC: $40.75
Published on Web 03/26/2009

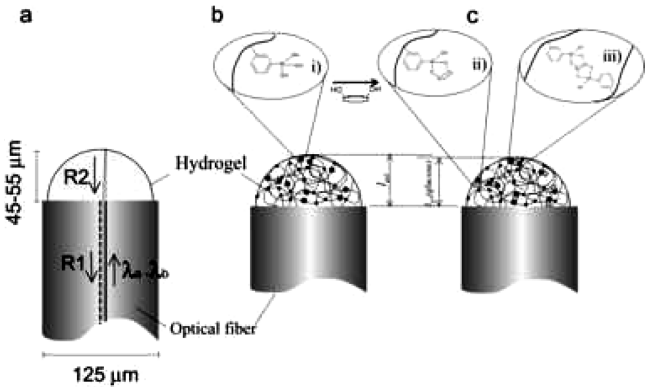

Figure 1. Schematic illustration of the principle for determination of changes in the optical length of the half-spherical biosensitive hydrogel covalently bound at the end of an optical fiber (a) and glucose-induced changes in hydrogel swelling of the phenylboronic acid functionalized hydrogel (b and c). The incident light (wavelength range $\lambda_a - \lambda_b$ from 1530 to 1560 nm) sent through the optical fiber is reflected both at the fiber−gel and gel−solution interfaces, R1 and R2, respectively, and changes in properties of the interference wave due to hydrogel swelling form the basis for determination of swelling (a). Glucose-induced swelling of phenylboronic acid (PBA, illustrated as hexagons) functionalized hydrogel by glucose-mediated cross-linking of two PBA moieties and binding to PBA (b and c). Cis-diols may interact with the PBA (i and ii) or cross-linking two PBA moieties (iii) supporting in increased cross-link density and associated change in equilibrium swelling. The hydrogel covalently linked to the end of the optical fiber adopts a near half-spherical geometry with length of the order of 45−55 μm (optical length of 60−70 μm).

strategy and furthermore by Lowe's group[11,15−17] employing a holographic-based determination of changes in the hydrogels. Examples of their work include development of carbohydrate sensing materials with high resolution at low ionic strength employing recognition by boronic acid[10] and incorporation of boronic acid derivatives in the acrylamide-based embedding matrix of the colloidal crystals for improved glucose selectivity.[14] Unlike enzymatic sensors, phenylboronic acids do not react exclusively to glucose but to cis-diols in general. The reversibility of cis-diols interacting with phenylboronic acids supports the development of detectors for continuous glucose monitoring.

Employing a responsive hydrogel-based sensor as previously reported[18] the present paper extends the sensor characterization and application by more extensive exploration of reproducibility, reversibility, molecular interference, and ultimately, preliminary measurements in blood plasma. For this purpose, an acrylamide-based hydrogel is used as the sensing material platform. Glucose recognition is achieved by incorporation of boronic acid moieties, 3-phenylboronic acid, into the gel. The resulting so-called smart hydrogel adopts an equilibrium swelling volume depending on the glucose concentration (Figure 1). Dimethylaminopropylacrylamide, a tertiary amine, was also integrated into the gel matrix to enhance glucose sensitivity and selectivity.[18] The molecular mechanism of incorporating a flanking amino group has been suggested to stabilize the formation of the negative complex between glucose and phenylboronic acid.[8] To our knowledge, glucose-sensitive hydrogels employing phenylboronic acid and a flanking amino group were first reported by Shiino et al.[19] and have been further investigated more recently.[15,18,20,21]

Previously, the sensor was optimized for sensitivity and selectivity toward glucose in comparison to other monosaccharides,[18] but due to the generic nature of the chemical bond formed by boronic acids and cis-diols, the hydrogel is susceptible to interference from other analytes such as polysaccharides, glycoproteins, and lactate. In this context, the lactate response was explicitly investigated here due to the relatively high concentration present in blood. Furthermore, experiments were carried out in fresh blood plasma to further assess the sensor potential for in vivo experiments.

Readout of the swelling of the glucose-sensitive hydrogel was achieved by chemically binding the hydrogel to the end of an optical fiber, which in turn is connected to a detector emitting light of a bandwidth 1530−1560 nm (Figure 1).[18,22] The swelling degree is monitored by an interferometric fringe technique where changes in the optical length of the hydrogel are manifested by a change in the phase of the interference wave arising from light reflected at the fiber−gel and gel−solution interfaces.[22] This has proven to be a highly sensitive technique with a resolution of ~2 nm and a detection rate of 1 Hz. The employed half-spherical hydrogels at the end of the optical fibers were 60−70 μm in optical length.

EXPERIMENTAL SECTION

Materials. Chemicals for preparation of the gels were obtained as follow: acrylamide (AAM) (99%, Sigma), *N*,*N*-methylenebisacrylamide (BIS) (≥99.5%, Fluka), 1-hydroxycyclo-hexylphenylketone (99%, Aldrich), ethylene glycol (>99.5%, Fluka), *N*-(3-dimethyl-aminopropyl)-acrylamide (DMAPAA) (>95%, TCI Europe NV), 3-(trimethoxysilyl) propylmethacrylate (Sigma, >98%), dimethyl sulfoxide (DMSO) (99.9%, Sigma), squalane (99%, Aldrich), 3-aminophenylboronic acid monohydrate (≥98%, Aldrich), acryloylchloride (99%, Fluka), sodium dihydrogen phosphate (Fluka, ≥99.5%), and disodium hydrogen phosphate anhydrous (>99.5%, Fluka). Water (resistivity 18.2 MΩ/cm obtained using a Millipore water purification system) was used for all solutions. The additives to the aqueous solutions were obtained as sodium chloride (>99.5%, Fluka), and D(+)-glucose (Sigma, 99%), sodium lactate (>99%, Fluka), sodium citrate (Sigma, USP testing specifications), and using HCl (36.5−38%, Sigma) to, e.g., adjust the final pH.

A 30 wt % AAM and 2 mol % BIS stock solution was prepared by dissolving the appropriate amounts in a 20 mM phosphate buffer, pH 7.4, for synthesis.

Sensor Fabrication. The pregel solution (10 wt % AAM, and 1.7 mol % BIS, 8 mol % 3-PBA, and 10 mol % DMAPAA) was prepared by adding appropriate amounts of the monomer 3-acry-

(15) Horgan, A. M.; Marshall, A. J.; Kew, S. J.; Dean, K. E. S.; Creasey, C. D.; Kabilan, S. *Biosens. Bioelectron.* **2006**, *21*, 1838–1845.
(16) Sartain, F. K.; Yang, X. P.; Lowe, C. R. *Anal. Chem.* **2006**, *78*, 5664–5670.
(17) Sartain, F. K.; Yang, X. P.; Lowe, C. R. *Chem.—Eur. J.* **2008**, *14*, 4060–4067.
(18) Tierney, S.; Volden, S.; Stokke, B. T. *Biosens. Bioelectron.*, **2009**, *24*, 2034–2039.
(19) Shiino, D.; Murata, Y.; Kubo, A.; Kim, Y. J.; Kataoka, K.; Koyama, Y.; Kikuchi, A.; Yokoyama, M.; Sakurai, Y.; Okano, T. *J. Contolled Release* **1995**, *37*, 269–276.
(20) Worsley, G. J.; Tourniaire, G. A.; Medlock, K. E. S.; Sartain, F. K.; Harmer, H. E.; Thatcher, M.; Horgan, A. M.; Pritchard, J. *Clin. Chem.* **2007**, *53*, 1820–1826.
(21) Yang, X. P.; Pan, X. H.; Blyth, J.; Lowe, C. R. *Biosens. Bioelectron.* **2008**, *23*, 899–905.
(22) Tierney, S.; Hjelme, D. R.; Stokke, B. T. *Anal. Chem.* **2008**, *80*, 5086–5093.

lamidophenylboronic acid (PBA), synthesized as previously described,[18] DMAPAA, and 20 mM phosphate buffer to the AAM/BIS stock solution. The initiator, 1-hydroxycyclo-hexylphenylketone, dissolved in ethylene glycol (0.1 M) was added to a concentration 0.13 mol % of the monomer. DMSO was further added to facilitate dissolution of 3-PBA, 10 vol %, to yield a 10 wt % pregel solution which was filtered before use (Acrodisc syringe filter, 0.2 μm pore size).

The synthesis setup consisted of one optical fiber for binding the gel (gel-fiber), a second fiber used as a UV waveguide, and a drop of squalane where the synthesis takes place. Optical fibers were prepared as described earlier by chemically modifying the fiber surface for covalently binding the gel to the surface Si.[23] A drop of the squalane solution was deposited on a glass rod wherein the gel-fiber was located, and an aliquot of the pregel solution was transferred to the end of the gel-fiber by a pipet. The gel-fiber was aligned with the UV waveguide (Dymax Medicure MC-4000) by optical stages and cured for 180 s, immersed briefly in pentane to remove the squalane, and subsequently placed in a phosphate-buffered saline solution, (PBS, 20 mM phosphate, 138 mM NaCl, pH 7.4) for 1 day to remove impurities.

Blood Plasma Preparation. Fresh blood was extracted from a healthy donor, into both ethylenediaminetetraacetic acid (EDTA) and heparinized blood glasses (BD vacutainer systems, Beliver Way Plymouth U.K.) and incubated at 37 °C for 24 h to glycolyse the blood.[24] The glucose concentration in blood after this treatment was determined with a hemoque glucose 201+ instrument verifying the glucose consumption. Subsequently, the sample was centrifuged (2800 rpm, 20 °C, for 10 min) and the supernatant was collected. The plasma was then left for at least 18 h exposed to the atmosphere to equilibrate with the atmospheric partial pressure of CO_2 (pCO_2), and a portion of the plasma was further centrifuged at 10 000 rpm through a 10 or 30 kDa molecular weight cutoff (MWCO) filter (Microcon centrifugal filter). Afterward, the plasma was heated to 37 °C and the pH was adjusted (PHM92 laboratory) to pH 7.4 followed by filtering (0.2 μm pore diameter) for sterilization.

Determination of Solutes Employing a Responsive Hydrogel Sensor. Determinations of glucose in aqueous solvents (PBS) and blood plasma were conducted by adding aliquots of a 0.1 M glucose stock solution to the test solutions. Glucose was dissolved in the PBS solution for at least 24 h prior to experiments ensuring mutarotation was complete. The majority of experiments were conducted by adding appropriate amounts of glucose stock solution to the pre-equilibrated sensor in PBS or blood plasma. In contrast, the reversibility experiment consisted of dipping the sensor in solutions of varying glucose concentrations. In this case the sensor was located in a glass tube where capillary forces ensured that the gel did not dry when transferring the sensor to a new solution. In all experiments, the sensor was left until equilibrium as viewed by the software, i.e., constant phase. Furthermore, the sample cell was immersed in a thermostatted water bath for temperature control (±0.1 °C). Following the completion of an experimental series, the gels were washed in PBS until constant phase, indicating the gel matrix was regenerated.

(23) Cras, J. J.; Rowe-Taitt, C. A.; Nivens, D. A.; Ligler, F. S. *Biosens. Bioelectron.* **1999**, *14*, 683–688.
(24) Chen, C. Y.; Ishihara, K.; Nakabayashi, N.; Tamiya, E.; Karube, I. *Biomed. Microdevices* **1999**, *1*, 155–166.

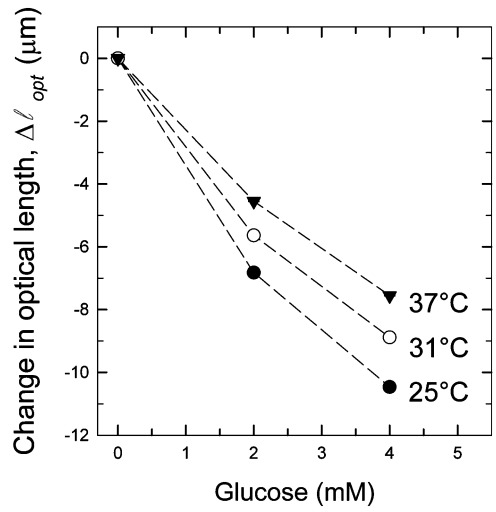

Figure 2. Change in the optical length of the gel, 8 mol % 3-PBA and 10 mol % DMAPAA, as a function of the glucose concentration (0−4 mM) in phosphate-buffered saline at 25 (●), 31 (○), and 37 °C (▼). All measurements were carried out twice and performed with pH = 7.4, I = 155 mM.

The instrument setup and detection method are basically as previously described.[22] However, an alternative detector was used with three channels compared to one channel as previously employed, thus enabling detection of three sensors simultaneously. The primary parameter, the optical length, is detected from the interference signal due to reflection of the light at the fiber−gel and gel−solution interfaces. Because of the low reflectivity of the gel−solution interface multiple reflections can be neglected, and the intensity signal can then be approximated by

$$I(\lambda) \approx I_0(\lambda)\left[r_1^2 + \gamma^2 r_2^2 + 2\gamma r_1 r_2 \cos\left(\frac{4\pi l_{\mathrm{opt}}}{\lambda} + \varphi_0\right)\right] \quad (1)$$

Here I_0 is the intensity of the incident light, r_1 and r_2 are the reflectivities at the fiber−gel and gel−solution interfaces, respectively, γ is the (wavelength and cavity length dependent) loss factor accounting for absorption, scattering, and mode mismatch, l_{opt} is the optical length of the gel cavity, λ is the wavelength, and φ_0 is the initial arbitrary phase. The observed changes in l_{opt} may originate both from changes of the physical length of the gel (l) and the refractive index (n_{gel}) of the gel, and a change in the optical length,Δl_{opt}, can be expressed as

$$\Delta l_{\mathrm{opt}} \approx \Delta l n_{\mathrm{gel}} + l\Delta n_{\mathrm{gel}} \quad (2)$$

n_{gel} depends both on the refractive index of the solution and polymer concentration, i.e., swelling degree of the gel. The uncertainties in the determination of Δl_{opt} for equilibrium swelling were estimated as the standard deviation from 20 points in at least two independent time series. The graphical representation of the standard deviations was generally smaller than size of the employed graphically symbols.

RESULTS AND DISCUSSION

Glucose-Induced Hydrogel Response at Various Temperatures. Figure 2 depicts the equilibrium swelling degree for the hydrogel at the three temperatures, 25, 31, and 37 °C, respectively. The data shows that the degree of glucose-induced swelling of the gel is reduced when the temperature is increased, with a

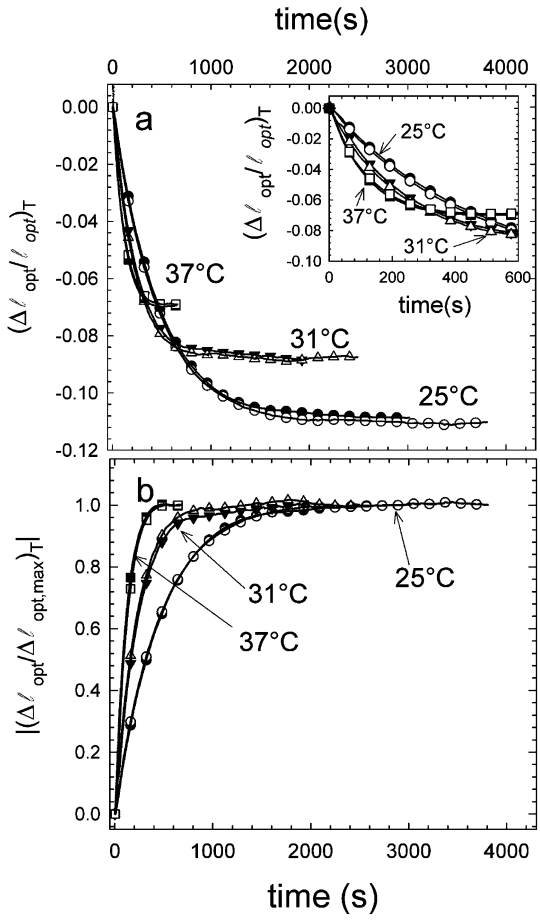

Figure 3. Kinetics of sensor response to a 2 mM increase in glucose concentration in phosphate-buffered saline of a 8 mol % 3-PBA and 10 mol % DMAPAA hydrogel at 25, 31, and 37 °C. The data are presented as the relative swelling of the hydrogel in phosphate-buffered saline at the actual temperature (a). The inset in panel a shows the initial change of the optical length. Every data point is depicted with small symbols, and large symbols are used for every 250 and 100 data points, panel a and panel a, inset, respectively. Normalized sensor response by the total change of the optical length at the actual temperature (b). Every data point is depicted with small symbols, and large symbols are used for every 250 data points.

reduction of glucose-induced Δl_{opt} approximately of 15−20% per 6°. The reduced value of Δl_{opt} encountered on increasing temperature does not, however, represent a practical limitation for the detection of the glucose concentration provided calibration is performed at the same temperature. Furthermore, with a resolution of ∼2 nm, detection of physiological glucose levels (4−8 mM),[25] is easily achieved.

The glucose-induced swelling was more rapid with increasing temperature. The data of $\Delta l_{opt}/l_{opt}$ obtained at 25, 31, and 37 °C (Figure 3, parts a and b) for the response due to 2 mM added glucose in the PBS buffer indicate the more rapid swelling at 37 °C compared to the lower temperatures. As previously reported,[18] at room temperature (∼20 °C), the response was characterized to being slow, and it had been previously reported of a 5-fold increase when going from 20−37 °C.[13] A simple phenomenological exponential model (one parameter) was used for determination of the hydrogel swelling kinetics induced by adding 2 mM glucose

(25) Boeckxstaens, G. E.; Horowitz, M.; Bermingham, H.; Holloway, R. H. *Neurogastroenterol. Motil.* **1997**, *9*, 239–246.

Table 1. Experimentally Determined Changes in Optical Length of Phenylboronic Acid Hydrogel Induced by Various Glucose Concentrations

temp °C	optical length change (μm) 2 mM glucose	optical length change (μm) 4 mM glucose	$t_{1/e}$ 10^2 s 0−2 mM glucose
25	6.8	10.5	4.5
31	5.6	8.9	2.2
37	4.5	7.6	1.2

to the PBS, at the various temperatures (Table 1). The data shows that the characteristic time to obtain 1/e of the total change in Δl_{opt} (2 mM glucose) is reduced by 50% by a 6 °C increase. Thus, the increase in temperature from 25 to 37 °C yields a total 4-fold increase in the swelling kinetics. The magnitude of Δl_{opt} for the same step change in glucose by increasing the temperature from 25 to 37 °C reduces the sensitivity by about 35%. Additional experiments were carried out at 39 °C (results not shown) where the similar results were seen, i.e., sensitivity change by 2.5% deg^{-1} and decrease in the time constant characterizing the kinetics by ∼17% deg^{-1}. Additionally, the length of the hydrogel increases with increasing temperature. This is in contrast to the theory of rubber elasticity which is usually employed on hydrogel swelling,[26,27] and may be due to solvent compatibility. Similar temperature-induced expansion as observed here has been reported previously for charged acrylamide-based hydrogels.[28,29] Although this effect could be of importance in understanding the temperature sensitivity of the glucose response, e.g., due to larger average distance between boronic acid groups with increasing temperature leading to a reduced amount of glucose-mediated cross-links between boronic acid, it does aid in explaining the kinetic effect. The main effect of the temperature on the sensitivity and kinetics of the glucose-induced change of Δl_{opt} is probably more likely to be due to the temperature effect of the chemical reaction between the boronic acid and glucose. Furthermore, the kinetics of the ionic strength induced changes in Δl_{opt} of about 2 s for a polycationic gel[22] suggest that the characteristic time constants observed here of 100 s and larger are limited by the glucose binding more than the kinetic response of the gel network itself. This is additionally highlighted in an experiment where the gel was exposed to sodium citrate (2 mM). The characteristic time is ∼16 s, much faster than the equivalent experiment with glucose.

Reversibility of Glucose-Induced Hydrogel Swelling. Reversibility is imperative for a sensor to be a serious candidate in glucose detection. In Figure 4 a sensor is exposed to stepwise increasing levels of glucose concentrations (0−2−4−8 mM) added to PBS. Following the exposure to the 8 mM glucose in PBS, the experiments proceeded by detecting the response to stepwise decreasing concentrations of glucose (8−4−2−0 mM). The data (Figure 4) indicate that the glucose-induced hydrogel swelling is a reversible process. Furthermore the kinetics are similar for both increasing and decreasing concentrations of glucose (∼100−140

(26) Treloar, L. R. G. *The Physics of Rubber Elasticity*; Clarendon Press: Oxford, U.K., 1975.
(27) Shibayama, M.; Tanaka, T. *Adv. Polym. Sci.* **1993**, *109*, 1–62.
(28) Caykara, T.; Akcakaya, I. *J. Appl. Polym. Sci.* **2007**, *104*, 2140–2145.
(29) Isik, B.; Dogantekin, B. *J. Appl. Polym. Sci.* **2005**, *96*, 1783–1788.

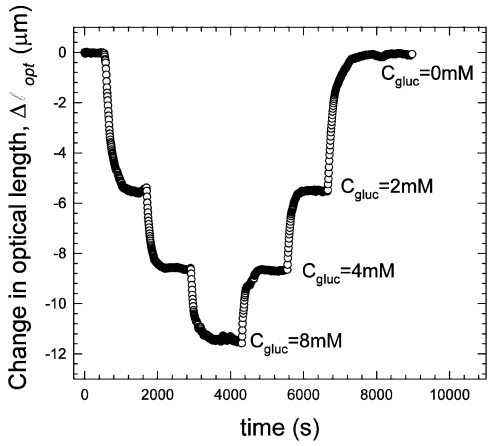

Figure 4. Change of optical length of 8 mol % 3-PBA and 10 mol % DMAPAA hydrogel vs time induced by stepwise increasing and decreasing glucose concentrations in phosphate-buffered saline. The glucose concentrations are indicated at the determined equilibrium lengths of the hydrogel. The measurements were carried out at 37 °C, pH = 7.4, and ionic strength of 155 mM.

s), although due to the experimental setup of these particular measurements, the characteristic times are more uncertain than other experiments performed.

Glucose-Induced Hydrogel Swelling with Different Sizes. The currently implemented manual deposition of the pregel solution on the end of a fiber via pipet leads to variations in hydrogel size. Although an automated procedure with a potential smaller variation in absolute size of the deposited gel would have been preferred, possible effects of absolute size appear not to be a serious limitation from the applied point of view. For the sensors in this project, the optical length of hydrogel varied between 62 and 71 μm when determined in PBS buffer. It is desirable to be able to use the sensor without calibration for every gel. Figure 5 shows an experiment where two gels, synthesized at approximately the same time, are exposed to stepwise increases in the glucose concentration in the range of 0−10 mM. The hydrogels immersed in the same solution, approximately 1 cm apart, are by their fiber-optic light-guide connected to the same three-channel detector. The optical lengths of the hydrogels, 64.2 and 70.5 μm, respectively, represent the maximum size difference for the gels fabricated. The data shows that this variation in size of the gels does not significantly affect their responses to glucose. In fact, the relative swelling of the two hydrogels, $\Delta l_{opt}/l_{opt}$, as induced by glucose, shows an almost identical performance independent of the size. This is the case for the both equilibrium swelling and the kinetic response. The difference in the relative equilibrium swelling degree between the two gels is in the order of 0.5−1%. In addition to showing that the size variation of about 10% is no major limitation, this data indicate excellent reproducibility for the gels synthesized. Furthermore, in terms of size, this means the gels do not need to be calibrated individually.

Molecular Interference with Sensitivity to Glucose. Boronic acids are known to react with cis-diols,[8] making it potentially reactive to a variety of components in blood that may interfere with determination of glucose. In particular, glycoproteins and lactate are potential response inhibitors to glucose. On the one hand glycoproteins are low in concentration in blood, but one

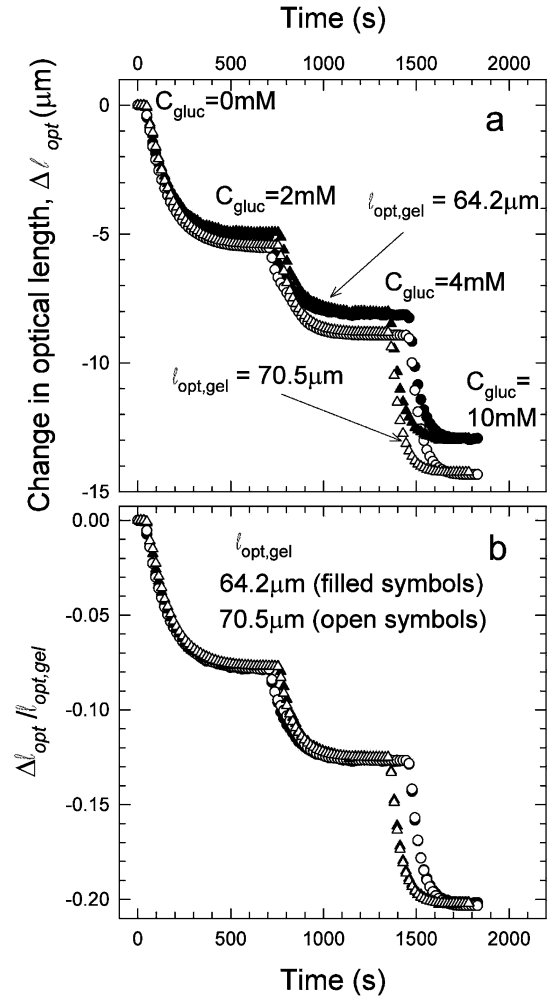

Figure 5. Optical response of two gels of varying size to glucose (0, 2, 4, 10 mM). The optical lengths of the gels are, respectively, 64.2 and 70.5 μm. (b) Same measurements as in panel a, but here the optical response is normalized by the optical length of each the gel. Measurements performed at 37 °C, pH = 7.4, and pI = 155 mM.

molecule could bind to multiple boronic acid molecules due to the many sugar groups on the protein. Lactate is also known to bind to phenylboronic acids.[16] Other sugars such as fructose, mannose, etc. are not expected to make much of an impact due their low concentrations in blood;[7] various monosaccharides have been investigated previously.[18]

Figure 6 depicts determination of glucose in PBS and subsequently with the same sensor for glucose determination in PBS with sodium lactate added to 1 and 5 mM, respectively. In comparison to the control experiment, there is a loss in sensitivity of ~4% with 1 mM lactate present, whereas at 5 mM, the sensitivity is reduced by ~17%. The ambient level of lactate in humans is approximately 1 mM,[30,31] although it can rise to much higher levels, for example, during exercise.[32] The pH of the solution was

(30) Csoregi, E.; Quinn, C. P.; Schmidtke, D. W.; Lindquist, S. E.; Pishko, M. V.; Ye, L.; Katakis, I.; Hubbell, J. A.; Heller, A. *Anal. Chem.* **1994**, *66*, 3131–3138.
(31) Feneberg, R.; Sparber, M.; Veldhuis, J. D.; Mehls, O.; Ritz, E.; Schaefer, F. *J. Clin. Endocrinol. Metab.* **1999**, *84*, 220–227.
(32) Shimojo, N.; Naka, K.; Uenoyama, H.; Hamamoto, K.; Yoshioka, K.; Okuda, K. *Clin. Chem.* **1993**, *39*, 2312–2314.

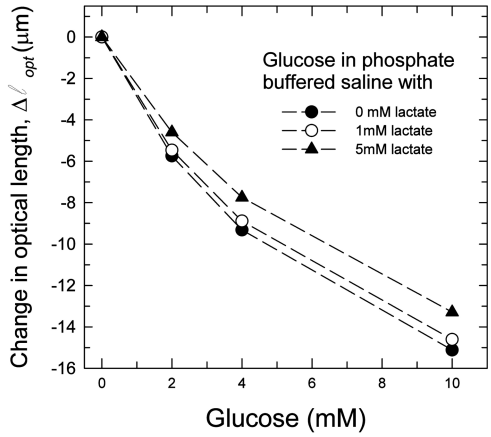

Figure 6. Response of 8 mol % 3-PBA, 10 mol % DMAPAA hydrogel to glucose, glucose with 1 mM lactate present, and glucose with 4 mM lactate present in 155 mM PBS at 37 °C, pH 7.4.

measured independently, which remained constant when 5 mM lactate was added.

Determination of various components in ex vivo blood may potentially be affected by the anticoagulants used. Blood delivered from blood banks is most commonly treated with a citrate-based solution, e.g., citrate phosphate dextrose solution. The concentration of citrate present in preserved blood is in the order of 15 mM (Baxter Healthcare Inc.). Measurements were conducted with sodium citrate where, for a 2 mM citrate addition, the equilibrium response was approximately 85% of the equivalent amount of glucose. Kinetically, however, the reaction was much faster with a $t_{1/e}$ of 16 s. It is believed that the slight increase in ionic strength is not the major contributor to the gel response; rather it would appear that citrate induces the formation of cross-links between PBA analogous to glucose. Citrate has been reported to bind with boronic acids.[33] In the concentration region used as an anticoagulant (15 mM), the glucose sensitivity was severely retarded (results not shown).

Commercial vacutainers, on the other hand, can be coated by a variety of different anticoagulants. Tests were conducted employing heparin- and EDTA-coated vacutainers to determine the potential interference from these chemicals when determining glucose in blood. PBS was added to a vacutainer and left for a couple of hours prior to testing. These experiments should be considered as crude guidelines because (a) the anticoagulants will bind in blood and (b) blood sample volumes will vary. For the PBS added to the heparin-containing vacutainer, Δl_{opt} was observed to about two-thirds relative to that in PBS. Immersion of the pre-equilibrated sensor into the heparin-containing solution yielded a slow change of Δl_{opt} indicative of large molecules binding to the gel. Heparin is a polysaccharide with multiple hydroxyl groups that can bind to the boronic acid moieties within the gel or on the surface. The glucose detection was, as expected, limited in this solution and kinetically a much slower response than in PBS. The most probable cause is due to competitive displacement of heparin by glucose. In addition, the hydrogel sensing element did not return to its original size following subsequent immersing in PBS. PBS stored in EDTA-containing vacutainers, on the other hand, did not induce any change in Δl_{opt}, provided the pH was adjusted. As the vacutainer was coated with K_2EDTA, a decrease in pH was expected. Moreover, the glucose response was the same here as in PBS. The structure of EDTA, with its four hydroxyl groups, could potentially bind with 3-PBA forming cross-links in hydrogel similar to citrate. The reason for the lack of EDTA-mediated cross-linking of the PBA groups is probably due to a larger distance between the hydroxyl groups in EDTA in comparison to citrate and carbohydrates.

In Vitro Determination of Glucose in Blood. As mentioned above, there are potentially interfering components that need to be taken into consideration when measuring blood-glucose variations ex vivo using the PBA-functionalized hydrogels. The choice of anticoagulant is one factor; additionally, the pH of blood becomes more alkaline due to the difference in carbon dioxide concentration in vivo and in vitro. Following the equilibration with the atmospheric partial pressure of CO_2 (18 h), the blood pH increased to 8.5−8.6. The plasma was therefore adjusted to pH 7.4 by a sterile sample of HCl solution to suppress the potential pH effect on the determination of glucose with the hydrogel sensor. Both EDTA- and heparin-coated vacutainers were used for blood collection. Due to the determined interference of heparin (see above), the plasma was centrifuged with a 10 000 molecular weight cutoff (Microcon centrifugal filter). The sensor response Δl_{opt} to glucose in this solution was about −0.33 of that in PBS. There was also a small decrease in sensor size on immersion into this solution. This is reminiscent to the shrinking induced in the heparinized PBS solution, but smaller in magnitude. Similar experiments were conducted where EDTA was used as the anticoagulant. In this case the plasma was centrifuged with a 30 000 MWCO. The data (Figure 7) shows that the sensor responds well to the glucose added to plasma in this case. Furthermore, the difference between the sensor in PBS and plasma is greatly reduced in comparison to heparin. The largest difference, about 15% in Δl_{opt}, is observed at the lowest concentration of glucose, 2 mM added to PBS and blood plasma, respectively (Figure 7b). The difference in the determined Δl_{opt} induced by added glucose to the two media decreases monotonically with further increase of glucose and is observed to 4% at 15 mM glucose (Figure 7b). The most probable cause is that there is a certain amount of interfering molecules present, e.g., lactate, which are eventually competitively displaced by glucose. The same trend was seen for the measurements where lactate was added to the solutions. The kinetics of the hydrogel swelling for a stepwise increase from 0 to 2 mM glucose was identical for plasma and PBS. Experiments were also conducted in untreated plasma, i.e., plasma not centrifuged with a MWCO filter, with EDTA as the anticoagulant. There was also a good response to glucose in this experiment, but some transient effect possibly from proteins in this solution was also observed. The nonlinear dependence of Δl_{opt} on glucose concentration, similar to our previous report,[18] requires a calibration taking into account this effect both in PBS and plasma.

The present experiments, indicating only a minimal amount of interference from foreign species, suggest that the PBA−DMAPAA sensing gel in combination with an optical readout have potential as a sensor for continuous measurements in blood. Although the plasma was centrifuged prior to use, the sensor would most likely

(33) Bosch, L. I.; Fyles, T. M.; James, T. D. *Tetrahedron* **2004**, *60*, 11175–11190.

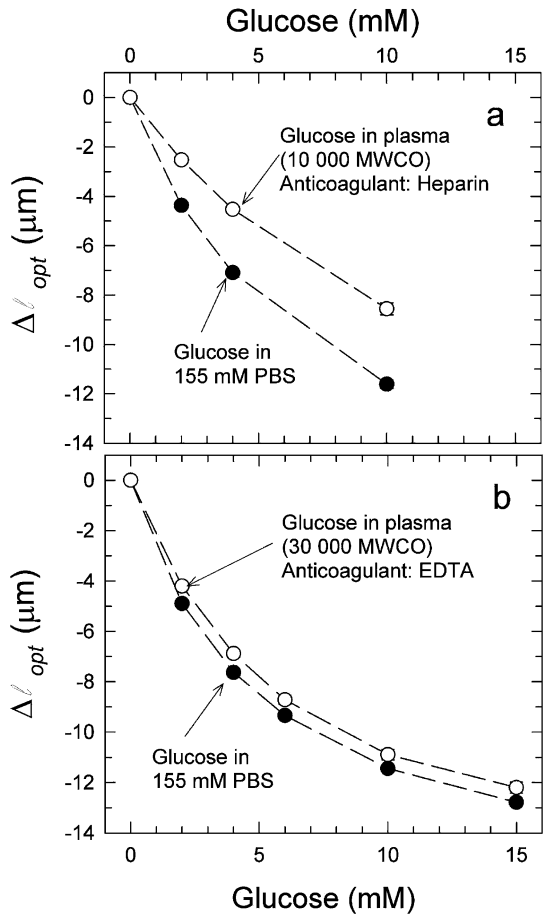

Figure 7. Response of 8 mol % 3-PBA, 10 mol % DMAPAA hydrogel to glucose, 0−15 mM, in 155 mM PBS and in pH-adjusted blood plasma. Experiments were carried out at 37 °C, pH = 7.4.

be encased in a membrane to avoid clotting factors. There are membranes available with pore sizes which coincide with that used here (MWCO 15 000 g/mol). The paramount feature of glucose sensors is to determine when blood-sugar levels are outside the normal levels of 4−8 mM, and with the resolution of this sensor, that is easily detected. Furthermore, the rapid glucose response, as supported by the size of the sensing hydrogel in the range of 50 μm, supports nearly real-time measurements. Future experiments will be conducted in blood plasma of varying concentrations to assess the long-term effects in blood and finally in vivo measurements.

CONCLUSION

A glucose-sensitive sensor was prepared by incorporation of 3-phenylboronic acid and dimethylaminopropylacrylamide into a hydrogel covalently linked to the end of an optical fiber. Various experiments were conducted to assess the sensor for continuous in vivo glucose monitoring. The sensor proved to be temperature-dependent, showing a 4-fold more rapid response, $1/t_{1/e}$, in the temperature interval from 25 to 37 °C. An associated temperature-dependent equilibrium hydrogel swelling of about 35% in the same temperature range was observed. This does not, however, pose any limitations due to the sensor resolution as long as the sensor is calibrated at the corresponding temperature. No hysteresis was seen when exposing the sensor to increasing and decreasing glucose concentrations, and the kinetics were very similar on both increasing and decreasing glucose concentrations in the interval of 0−8 mM. The results also showed that there was negligible interference for glucose measurements at normal blood-lactate levels (1 mM), although increased interference at an elevated lactate concentration (5 mM) cannot be neglected. Anticoagulants often used for blood storage, i.e., heparin and citrate, proved to interfere with the determination of glucose using the sensor, and it was found that EDTA was the best suited anticoagulant for the experiments in blood plasma in vitro. The sensor showed a very good response to glucose variations in ex vivo blood plasma that had been initially collected with EDTA as the anticoagulant, with a 15% to 4% reduction compared to the determination in PBS. The interference from foreign analytes was largest at low concentrations and decreased as the glucose concentration increased. Kinetically, the measurements in plasma and PBS were analogous. These results show good promise for the sensor to be applied as an in vivo glucose monitoring system and will be further investigated in continuous plasma experiments.

ACKNOWLEDGMENT

This work was supported by the strategic university program in medical technology at NTNU, project no. 154080 supported by the Norwegian Research Council.

Received for review January 5, 2009. Accepted February 23, 2009.

AC900019K