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Measurement of Glucose in Blood with a Phenylboronic Acid Optical Sensor

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Abstract

Background:

Current methods of glucose monitoring rely predominantly on enzymes such as glucose oxidase for detection. Phenylboronic acid receptors have been proposed as alternative glucose binders. A unique property of these molecules is their ability to bind glucose in a fully reversible covalent manner that facilitates direct continuous measurements. We examined (1) the ability of a phenylboronic-based sensor to measure glucose in blood and blood plasma and (2) the effect on measurement accuracy of a range of potential interferents. We also showed that the sensor is able to track glucose fluctuations occurring at rates mimicking those experienced *in vivo*.

Method:

In vitro static measurements of glucose in blood and blood plasma were conducted using holographic sensors containing acrylamide, *N*,*N*′-methylenebisacrylamide, 3-acrylamidophenylboronic acid, and (3-acrylamidopropyl) trimethylammonium chloride. The same sensors were also used for *in vitro* measurements performed under flow conditions.

Results:

The opacity of the liquid had no affect on the ability of the optical sensor to measure glucose in blood or blood plasma. The presence of common antibiotics, diabetic drugs, pain killers, and endogenous substances did not affect the measurement accuracy, as shown by error grid analysis. *Ex vivo* flow experiments showed that the sensor is able to track changes accurately in concentration occurring in real time without lag or evidence of hysteresis.

Conclusions:

The ability of phenylboronic acid sensors to measure glucose in whole blood was demonstrated for the first time. Holographic sensors are ideally suited to continuous blood glucose measurements, being physically and chemically robust and potentially calibration free.

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Abbreviations: (3-APB) 3-acrylamidophenylboronic acid, (ATMA) (3-acrylamidopropyl) trimethylammonium chloride, (CLSI) Clinical and Laboratory Standards Institute, (EDTA) ethylenediaminetetraacetic acid, (MBA) *N*,*N*′-methylene bisacrylamide, (PBS) phosphate-buffered saline, (YSI) Yellow Springs Instruments

Keywords: blood, continuous, diabetes, glucose, optical, phenylboronic acid, sensor

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Inroduction

With diabetes mellitus reaching pandemic proportions, alternative approaches of glucose monitoring are being actively developed to overcome the limitations of existing enzyme-based electrochemical methods. One method with potential to provide more reliable long-term continuous monitoring uses the group of synthetic receptors called phenylboronic acids.[1–4] These Lewis acids can bind the *cis*-1,2- or -1,3-diols of glucose covalently to form five- or six-membered rings. Unusually, the binding of saccharides is reversible, allowing a direct measurement of glucose in contrast to enzyme-based methods. A further advantage of these compounds is that glucose is not consumed in the measurement process nor are reaction products generated that may deleteriously affect the long-term repeatability of measurements made by the sensor.

We demonstrated for the first time that a phenylboronic acid-based sensor can function in blood plasma.[5] This is as opposed to simple buffers with selected additives, e.g., sodium chloride, proteins, and so on.[6–8] Herein, we examine the ability of the sensor to measure glucose in blood and demonstrate for the easier medium of blood plasma (in terms of handling *ex vivo*) that the sensor can track changes in glucose occurring in real time at rates mimicking those experienced *in vivo*. We also demonstrate the effect of different substances (e.g., common pain killers, antibiotics, blood preservatives, antidiabetic drugs, blood thinners, and blood metabolites) on measurement accuracy.

To measure glucose, we use a novel optical technique first described by Lowe and co-workers wherein phenylboronic acid receptors are immobilized in a hydrogel containing a holographic grating.[9,10] Binding of glucose to the receptors induces a change in the swelling state of the hydrogel, increasing or decreasing the spacing between the holographic fringes of the grating, in turn modulating the wavelength of light that is diffracted in a manner determined by Bragg's law. The advantages of this detection method over other optical techniques such as fluorescence are the stability of the sensor and the ease with which the wavelength may be tuned to suit the application. The incorporation of phenylboronic acid receptors enables these format-flexible sensors to operate continuously for extended periods without needing recalibration, providing an attractive alternative to conventional electrochemical enzyme-based methods of glucose monitoring for people afflicted with diabetes mellitus.

Materials and Methods

The materials and equipment used to make the holograms and the materials for the buffer solutions are described elsewhere.[11] Human blood plasma (single donor and pooled) was purchased from Scipac Ltd. (Kent, UK), Patricell Ltd. (Nottinghamshire, UK), Cambridge Biosciences Ltd. (Cambridgeshire, UK), and Valley Biomedical (Winchester, Virginia). Horse blood was purchased from TCS Biosciences (Buckingham, UK). Samples were supplied with either Na ethylenediaminetetraacetic acid (EDTA) or K_3 EDTA as the anticoagulant. Horse blood was used fresh. Plasma samples were aliquoted and stored at –20°C upon receipt.[12,13] In accordance with guidelines, plasma samples were thawed from frozen through incubation at 37°C in a water bath to prevent cryoprecipitation.[14] Measurements of plasma sample pH were obtained with a Hanna Instruments pH 213 Microprocessor pH meter or an ELIT eight-channel pH analyzer (NICO2000 Ltd., Middlesex, UK) equipped with a Hamilton Biotrode (Reno, Nevada) or a Jencons P 16 (Bedfordshire, UK) microelectrode designed for use in small volume proteinaceous samples. Measurements of blood sample pH were obtained with a Hamilton Double Pore electrode. The pH microelectrodes were calibrated with a two-point calibration using fresh 20-ml sachets of ready-mixed National Institute of Standards and Technology traceable pH buffer from Hanna Instruments (Bedfordshire, UK) at pH 7.01 and 10.01 prior to measurement. Ismatec Reglo digital ISM 834 peristaltic pumps (Glattbrugg, Switzerland) fitted with Tygon tubing (Ismatec SC0061 and SC0068 set for flow rates of 78.4 and 175 µl/min, respectively) were used for glucose tracking experiments.

Sensor Hologram Preparation

The following holograms were prepared on glass microscope slides as described elsewhere[11]: 3 mol% *N*,*N*′-methylene bisacrylamide (MBA), 12 mol% 3-acrylamidophenylboronic acid (3-APB), and 12 mol% (3-acrylamidopropyl)trimethylammonium chloride (ATMA).

Blood and Plasma Sample preparation and pH Control

Ex vivo blood and blood plasma become more alkaline when exposed to the atmosphere due to the continual loss of carbon dioxide and its role in the carbonic acid–bicarbonate buffering mechanism.[15] To limit the effects of sample pH fluctuations during use and to mimic conditions *in vivo*, two methods for pH control were used. The method used to create plasma samples

for glucose tracking experiments required buffering the plasma within the physiological pH range with sodium phosphate, producing a final addition of 0.1 *M* (19 mol% monobasic to 81 mol% dibasic). The buffered solution was then exposed to the atmosphere for 16 hours to equilibrate pCO_2 before heating to 37°C and measuring the final pH. The sample was subsequently sterilized by passing through a 0.45-µm Sartorius Minisart High Flow syringe filter (Goettingen, Germany). In the second method used for static cell experiments involving blood and plasma samples, CO_2 was infused directly into the sample using a syringe to lower the sample pH to pH 7.20, and the diffraction wavelength of the hologram was read when the sample pH attained pH 7.40. Commercially available human plasma is supplied typically within the normal range of glucose concentrations for adults (74–106 mg/dl).[13] To create a panel of samples covering the pathological range of glucose concentrations, blood and plasma samples were spiked with additional glucose from a concentrated plasma or blood stock. Samples were allowed to equilibrate for a minimum of 45 minutes before use in accordance with International Standards Organization guidelines to allow time for the mutarotation of glucose.[16] Plasma samples containing elevated levels of endogenous and exogenous substances were prepared in a similar way. In some cases (troglitazone, Warfarin), methyl sulfoxide was needed to aid dissolution at the specified concentration. 4-Acetamidophenol, acetylsalicylic acid, amikacin dihydrate, ascorbic acid, cefotaxime sodium salt, EDTA disodium salt dihydrate, D-(+)-galactose, warfarin, urea, L-glutathione (reduced), 2,5-dihdroxybenzoic acid (gentisic acid), 3-hydroxytyramine hydrochloride, all of purity greater than 98%, and heparin sodium salt (Dalteparin) were purchased from Sigma-Aldrich (Poole, UK). Tetracycline was purchased from Fluka (Poole, UK). Uric acid and creatinine of purity greater than 99% were purchased from Acros (Leicestershire, UK). Glibenclamide, metformin hydrochloride, miglitol, nateglinide, and troglitazone were purchased from Sequoia Research Products (Berkshire, UK). All chemicals were used as received.

Hologram Glucose Interrogation and System-Specific Sensor Calibration

Sensor holograms were interrogated from the glass substrate side as described elsewhere.[11] Sensor calibration was achieved through the comparison of a sensor diffraction wavelength with a sample glucose concentration measured using a Yellow Springs Instruments (YSI) 2300 STAT plus glucose analyzer (Yellow Springs, OH). The holographic sensor directly detects analyte molality (moles of analyte per volume of water) of the undiluted sample, and the YSI measures analyte molarity (moles of analyte per volume of solution) in a diluted sample. In the case of plasma measurements, calibration was performed using a plasma standard to decrease the systematic error produced through the difference in the water content upon the YSI measured glucose concentration, which would occur if calibrated in an aqueous buffer.[13,17]

Static Calibration

Each hologram slice was equilibrated in 1.5 ml phosphate-buffered saline (PBS) to establish a zero glucose baseline before removal of the PBS and the addition of 1.5 ml of plasma. Sample pH was manipulated as described earlier; after the sample attained pH 7.40, the sensor diffraction wavelength was measured and a 60-µl aliquot of plasma was removed from the cuvette for YSI analysis to establish the sample glucose concentration. A 60-µl aliquot of 0.1 *M* glucose in plasma was then added. The sample pH was then lowered, and this process was repeated until a glucose concentration over 540 mg/dl was recorded on the YSI. For blood measurements, sensors were calibrated in 1.5 ml PBS. At 45-minute intervals a 60-µl aliquot was removed for YSI analysis before the addition of a 60-µl aliquot of 0.1 *M* glucose in PBS. Nonlinear calibration data were fitted using the modified single three parameter exponential decay function in SigmaPlot 9.0 from Systat Software, Inc. (San Jose, CA).

Flow Calibration

The sensor cuvette was filled with 1.5 ml buffered blood plasma and a peristaltic pump primed with 1.7 ml buffered plasma. During the assay, plasma was circulated to and from the cuvette at a rate of 175 µl/min, mimicking the system under operation in a glucose tracking experiment. At 45-minute intervals a 60-µl sample of plasma was removed from the cuvette for analysis upon YSI and a 60-µl aliquot of 0.1 *M* glucose in plasma was added to the cuvette. Between each addition and removal the sensor was allowed to equilibrate to a stable diffraction wavelength. This was repeated until a 300-mg/dl glucose concentration was recorded, producing a calibration plot.

Ex Vivo Glucose Sensing

Hologram slices were equilibrated in 1.5 ml PBS before complete solution exchange into sample plasma (or blood). Sample pH manipulation was performed as stated earlier, and the sensor wavelength was recorded when pH 7.40 was attained. Simultaneously, the sample

glucose concentration was measured on the YSI. Whole horse blood samples were assumed to contain 84% water; accordingly, YSI glucose concentrations were corrected, enabling comparison with the PBS-calibrated sensor.[17] The plot equation function in SigmaPlot 9.0 was used to interpolate or extrapolate the sample concentration from the sensor response.

Holographic-Based Continuous Glucose Sensing in Plasma

Glucose tracking was achieved through a fluidic system using two peristaltic pumps. Both peristaltic pumps were primed and initialized 10 minutes before interrogation of the sample, enabling the system to equilibrate. The plasma glucose concentration in the cuvette was increased through the infusion of high glucose stock plasma (8.0 mg/ml) at a rate of 78.4 µl/min into a stirred plasma reservoir containing 15 ml of plasma. The reservoir had an initial theoretical rate of change of 4.18 mg/dl/min. The reservoir plasma was fed at a rate of 175 µl/min into the sensor cuvette, which was emptied to waste at a rate of 175 µl/min, retaining a constant cuvette volume of 1.5 ml. When the appropriate plasma glucose concentration was attained in the reservoir, the pump infusing high glucose stock plasma was stopped. The plasma glucose concentration within the cuvette was decreased through replacement of the reservoir every 8.5 minutes with a plasma sample containing a 25-mg/dl lower glucose concentration, aiming for a rate of change of 3 mg/dl/min. Throughout the procedure, 40-µl plasma samples were collected every 2 to 5 minutes from the cuvette for glucose measurement by YSI and the sensor diffraction wavelength was recorded. Following a complete cycle of glucose concentration, the sensor was washed into PBS for overnight storage. Daily glucose excursions in plasma were collected over a 3-day period without further calibration.

Hologram Sterilization

To determine if the holograms would support sterilization, which is necessary to remove harmful pathogens, test samples were sterilized with a calibrated Priorclave PS-QCS-SH150 (Woolwich, UK). A steam sterilization cycle of 121°C for 15 minutes was used following published guidelines.[18]

Results and Discussion

Figure 1 shows the signal from a 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA hologram in PBS and horse blood. When illuminated with ordinary white light, the holographic fringes within the gel collectively diffract a narrow band of wavelengths in a manner governed by the Bragg equation ($\lambda_{max} = 2nd \sin\theta$). The end result is a characteristic spectral reflection peak whose wavelength can be correlated with glucose concentration. The holograms used in this study replayed in the red to yellow region of the visible spectrum. The presence of glucose causes the diffraction peak to blue shift toward the yellow, corresponding to hydrogel contraction perpendicular to the fringe planes. Previously, we proposed that the contraction was caused by cross-linking of two boronic acid receptors with favorable stereochemistry by glucose to give a bis-boronate–glucose complex.[11] Results illustrate that the opacity of the liquid under investigation does not alter the ability to detect the optical signal and measure the wavelength of the diffracted light. The holograms were created using a Denisyuk setup and are examples of "reflection" holograms. They have the property that the signal can be viewed from the glass substrate side of the hologram with the opposite (polymer) face in contact with the liquid rather than the converse, monitoring through the liquid in transmission.

To demonstrate efficacy in blood, 20 measurements were made with the holograms and compared with YSI results after they had been corrected for hematocrit.[19] Data pairs are plotted in **Figure 2** in the form of a Clarke error grid, which separates the results into zones reflecting the clinical significance of the measurement error.[20] Eighteen out of 20 (90%) measurements are located in zone A (i.e., accurate), whereas 2 out of 20 (10%) measurements are located in zone B (i.e., benign outcome). There are no points in zones C–E corresponding to errors, which are potentially dangerous. It is anticipated

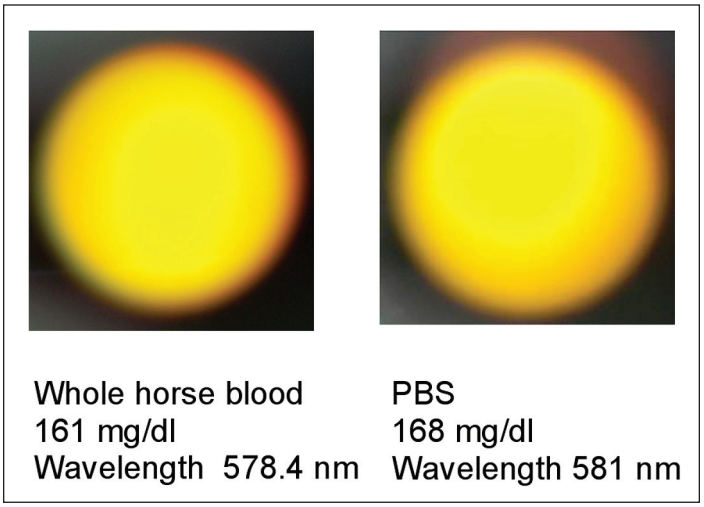

Figure 1. The color of a 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA hologram in whole (horse) blood and PBS at pH 7.40 and 37°C. The glucose concentrations of the samples were adjusted to be approximately the same so that they replay the same color (yellow).

that changes to the experimental system, such as clamping the hologram more tightly to prevent movement, will result in better measurement accuracy and precision so that the results are more tightly bunched around the 45° degree center line corresponding to perfect correlation between the two measurement methods. **Figure 3** compares the behavior of different hologram slices cut from different holograms prepared at different times by different people. Data have been normalized to allow for differences in starting wavelength due to the use of different hydrogel equilibration times in the hologram exposure bath.[11] Normalized data show that holograms of the same formulation behave extremely similarly and raise the possibility of being able to mass manufacture materials that do not require calibration before use. Samples that were steam sterilized at 121°C for 15 minutes gave slightly different calibration curves (data not shown). The starting wavelength was lower and they contracted slightly less. However, samples within the same batch were altered to exactly the same extent. From these results it can be inferred that the sensors are amenable to industrial scale-up and use in medical devices.

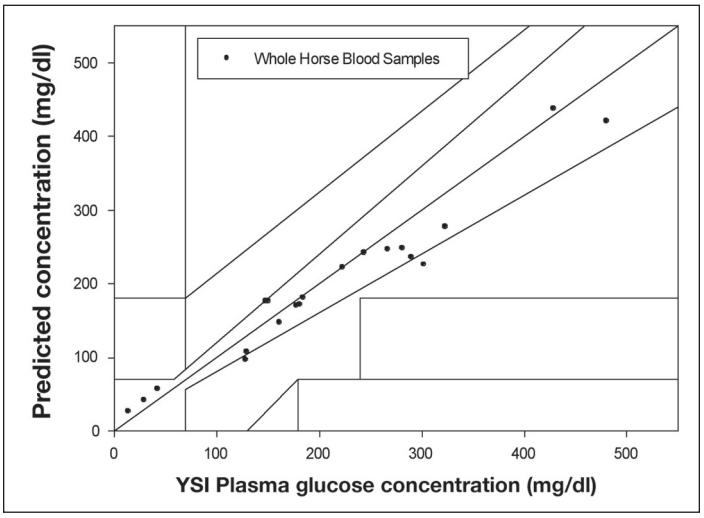

Figure 2. Measurements in whole (horse) blood using a 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA hologram at pH 7.40 and 37°C. Results are plotted against the corrected values for the YSI reference method and presented in the form of a Clarke error grid. The measurement reproducibility associated with the experimental test system was 5.4, 4.4, and 1.9% coefficient of variation at 72, 144, and 540 mg/dl, respectively. The measurement repeatability was 4.8, 1.1, and 1.5% coefficient of variation at 72, 144, and 540 mg/dl, respectively.

The ability of sensors to function in a real physiological liquid while in the presence of substances with the potential to interfere with the measurement was investigated using human plasma. Plasma was preferred to blood for this work as it is easier to handle and can be stored indefinitely under suitable conditions. The plasmas used are expected to have normal background levels of endogenous and exogenous substances. **Table 1** contains details of the substances used and their test levels. The list includes substances not normally found in the body, as well as substances that may occur in the blood at high concentrations due to a particular medication, disease state, or dietary regime. For most substances, Clinical and Laboratory Standards Institute (CLSI)-recommended test levels were used. Where unavailable, the therapeutic dose was assumed to be distributed in 5 liters of blood and was then tested at greater than three times this concentration.[21] Miglitol and natglinide concentrations were derived from the 27th Food and Drug Administration-approved drug products list.[22] For each substance tested, a measurement was usually made at three different glucose concentrations: low (~79–150 mg/dl), medium (~150–300 mg/dl), and high (~300–540 mg/dl). Results are expressed in the form of a Clarke error grid in **Figure 4**. Fifty-seven out of 63 (90.47%) measurements are located in zone A (i.e., accurate), whereas 6 out of 63 (9.5%) measurements are located in zone B (i.e., benign outcome). While the list of substances tested is not exhaustive, these preliminary results suggest that the sensor has good specificity for glucose in plasma and that the measurement method is robust. Future work will extend the number of samples to include other substances found in food supplements, administered in intensive care settings, or produced in pathological conditions as metabolites.

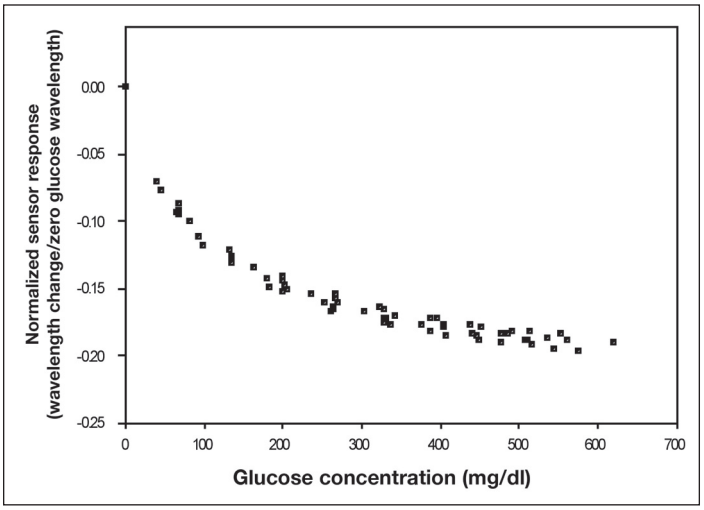

Figure 3. Normalized glucose in PBS calibration plots at pH 7.40 and 37°C for different slices of 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA holograms made on 4 different days and derived from three different hydrogel batches. Calibration data were normalized by dividing the diffraction peak shift at each concentration by the starting wavelength.

Table 1.
Substances Tested for Their Effect on Measurement Accuracy[a]

Interferent	Substance class	Test concentration	CLSI-recommended test concentration	Therapeutic/pathological concentration/reference interval
2,5-Dihydroxybenzoic acid	Anti-inflammatory agent	1.8 mg/dl	1.8 mg/dl	0.2–0.6 mg/dl
3-Hydroxytyramine hydrochloride	Hormone/ neurotransmitter	0.09 mg/dl	0.09 mg/dl	0.03 mg/dl
4-Acetamidophenol	Analgesic	20 mg/dl	20 mg/dl	1–3 mg/dl
Acetylsalicylic acid	Analgesic	65 mg/dl	65 mg/dl	13–39 mg/dl
Amikacin dihydrate	Antibiotic	8 mg/dl	8 mg/dl	1–4 mg/dl
Ascorbic acid	Vitamin	3 mg/dl	3 mg/dl	0.4–1.5 mg/dl
Cefotaxime	Antibiotic	60 mg/dl	30 mg/dl	4–10 mg/dl
Creatinine	Anaerobic by-product	5 mg/dl	5 mg/dl	0.6–1.3 mg/dl
Dalteparin	Anticoagulant	300 U/dl	300 U/dl	35–100 U/dl
EDTA	Anticoagulant	4 mg/dl	0.1 mg/dl	—
D-(+)-Galactose	Monosaccharide	20 mg/dl	15.1 mg/dl	<5.0 mg/dl
Glibenclamide	Antidiabetic drug	1.2 mg/dl	0.2 mg/dl	0.06 mg/dl
L-Glutathione (reduced)	Tripeptide	92 mg/dl	92 mg/dl	24.25–32.2 mg/dl
Metformin HCl	Antidiabetic drug	4 mg/dl	4 mg/dl	0.5–4 mg/dl
Miglitol	Antidiabetic drug	6 mg/dl	100-mg oral dose (6 mg/dl)[b]	—
Nateglinide	Antidiabetic drug	9 mg/dl	120-mg oral dose (7.2 mg/dl)[b]	—
Tetracyclin	Antibiotic	1.5 mg/dl	1.5 mg/dl	0.2–0.5 mg/dl
Troglitazone	Antidiabetic drug	0.9 mg/dl	0.9 mg/dl	0.1–0.3 mg/dl
Urea	Metabolic by-product	257 mg/dl	257 mg/dl	6.6–85.8 mg/dl
Uric acid	Metabolic by-product	23.5 mg/dl	23.5 mg/dl	2.5–8 mg/dl
Warfarin	Anticoagulant	5 mg/dl	1 mg/dl	0.1–0.3 mg/dl

[a]Clinical and Laboratory Standards Institute-recommended test levels were used. For each substance tested, a measurement was usually made at three different glucose concentrations: low (~79–150 mg/dl), medium (~150–300 mg/dl), and high (~300–540 mg/dl).
[b]Where unavailable, the therapeutic dose was assumed to be distributed in 5 liters of blood and then tested at greater than three times this concentration.[21,22]

To see if the sensors could track changes in glucose occurring in real time, a flow experiment was conducted wherein the glucose concentration was cycled up and down three times at approximately the maximum rate of change *in vivo*. The distribution of rates for the diabetic population was characterized previously by Dunn and colleagues.[23] Rates in excess of 3 mg/dl/min (upward and downward) are observed in less than 1% of observations while the majority of time blood glucose changes in the body are less than 1 mg/dl/min. A concern regarding hydrogels based on phenylboronic acids is their relatively slow response times. Often, rather than quoting equilibrium values, response times are given in terms of the time taken for the signal to reach 90% of its maximum.[24] The degree of correlation evident from **Figure 5** demonstrates that, contrary to perceptions, the response characteristics of phenylboronic acid-based hydrogels are perfectly adequate for applications where continuous monitoring is a benefit, for instance, in type 1 diabetes or to facilitate intensive insulin therapy in critical care settings.[25] With time intervals of 3 minutes, greater than 90% of the results fall within the accurate

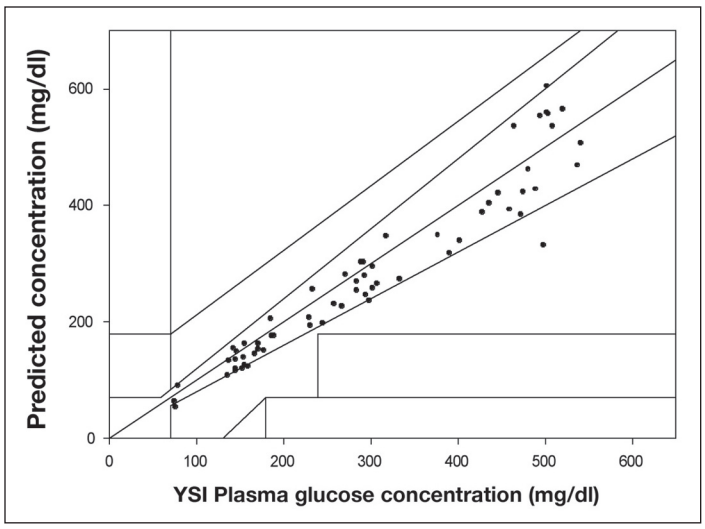

Figure 4. The measurement of glucose in human plasma samples containing different types of endogenous and exogenous substances using a 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA hologram at pH 7.40 and 37°C. Results are plotted against the true values obtained using the YSI reference method. Test levels of the substances are specified in **Table 1**.

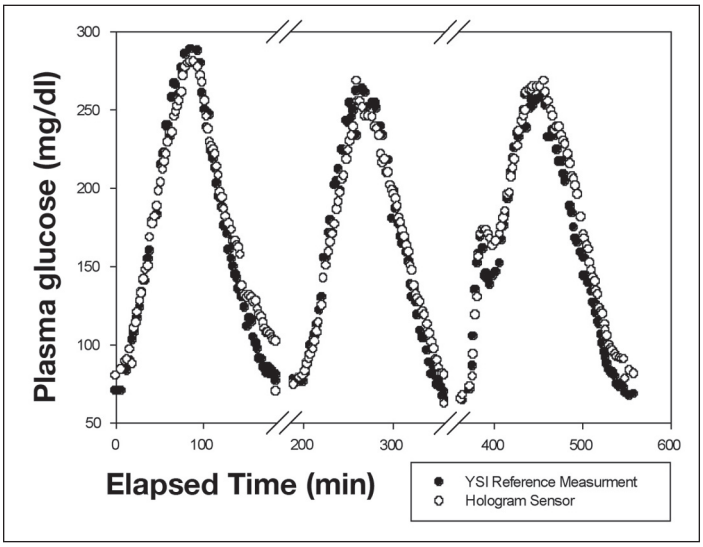

Figure 5. Consecutive glucose concentration versus time profiles obtained in human blood plasma at physiological pH and temperature (pH 7.40 and 37°C) with a 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA hologram. Hologram results were obtained in real time. YSI samples were collected in real time but were processed after the experiment to obtain the reference concentration versus time profile. Experimental intervals indicated by x axis breaks. At 145 minutes the sensor was knocked inadvertently, resulting in the jump in sensor concentration measurement due to the angular change.

region of a rate error grid, indicating a clinically acceptable lag between the sensor and the reference method.[5]

The ability of these holographic sensors to monitor in real time over long periods makes them ideally suited to continuous glucose monitoring. Typically, each holographic sensor in this study was used at 37°C for approximately 1 month to make multiple sample measurements *ex vivo*. The experiment to measure fluctuating glucose concentrations in **Figure 5** occurred over a 5-day period, about the length of time a hologram-based fiber optic might be used if implanted into the skin or a blood vessel. It is anticipated that the simplicity and biocompatibility of the sensor should lead to more reliable long-term monitoring.[26–28] As with conventional enzymatic methods of glucose monitoring, the robustness of the measurement will depend on the format of the final device (e.g., contact lens, fiber-optic catheter), the location of use (e.g., *in vivo*, *ex vivo*), and the precautions taken to prevent measurement error. Temperature, pH, and ionic strength effects should be minimized if used *in vivo*, as the body is able to control these variables within narrow ranges.[13] Accurate measurements with the current sensors were possible over the physiological temperature range and at pathological ionic strengths (250–325 mOsmol/kg) (data not shown). The measurements were slightly more sensitive to pH variation, which may be attributed to the pH dependence of the 3-APB receptor and its reasonably high pK_a.[29] Experiments have shown that the pH dependence may be negated by calibrating for pH and making a simultaneous pH measurement (data not shown). Holographic sensors have been described previously that provide very sensitive and robust measurements of pH.[30] In fact, a continuous blood pH measurement may be desirable in some applications, such as hospital-based patient monitoring. Future work will look at holographic sensors incorporating 2-acrylamidophenylboronic acid derivatives. These phenylboronic acids have an oxygen electron donor intramolecular to the boron and have been shown to exist in a zwitterionic tetrahedral form, which is insensitive to changes in pH.[31] The use of these receptors will remove the need outside the normal physiological pH range for a separate pH measurement to increase accuracy.

Conclusions

We have demonstrated that phenylboronic acid-based sensors can measure glucose in real biological fluids and track changes in concentration occurring at rates mimicking those *in vivo*. The glucose-sensitive holograms used in this study may potentially offer calibration-free measurements and more reliable continuous monitoring. The latter is of major importance in conditions such as type 1 diabetes and in critical care settings where hyperglycemia may lead to increased morbidity and mortality. Future work will examine the behavior of these sensors and versions thereof in the presence of a wider range of therapeutic substances under physiological conditions corresponding to different disease states.

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