Continuous Blood Glucose Monitoring with a Thin-Film Optical Sensor

Graham J. Worsley, Guilhem A. Tourniaire, Kathryn E. S. Medlock, Felicity K. Sartain, Hazel E. Harmer, Michael Thatcher, Adrian M. Horgan, and John Pritchard

Background: We recently described a holographic optical sensor with improved selectivity for glucose over fructose that was based on a thin-film polymer hydrogel containing phenylboronic acid receptors. The aim of the present work was to measure glucose in human blood plasma as opposed to simple buffers and track changes in concentration at a rate mimicking glucose changes in vivo.

Methods: We used holographic sensors containing acrylamide, N,N’-methylenebisacrylamide, 3-acrylamidophenylboronic acid, and (3-acrylamidopropyl)trimethylammonium chloride to measure 7 human blood plasma samples at different glucose concentrations (3–33 mmol/L) in static mode. Separately, using a flow cell, the glucose concentration was varied at approximately 0.17–0.28 mmol/L/min, and the sensor’s ability to continuously monitor glucose was investigated over an extended period.

Results: We subjected the results of the ex vivo static measurements to error grid analysis. Of 46 measurements, 42 (91.3%) fell in zone A of a Clarke error grid, and the remainder (8.7%) fell in zone B. The ex vivo flow experiments showed that the sensor is able to accurately track changes in concentration occurring in real time without lag or evidence of hysteresis.

Conclusions: We demonstrate the ability of a phenylboronic acid–based sensor to measure glucose in human blood plasma for the 1st time in vitro. Holographic glucose sensors can be used without recourse to recalibration. Their robust nature, coupled with their format flexibility, makes them an attractive alternative to conventional electrochemical enzyme-based methods of glucose monitoring for people with diabetes.

Conventionally, glucose is measured electrochemically via enzymes on an intermittent basis determined by patient compliance. Consequently, excursions into hyper- or hypoglycemia may be missed, with potential long-term health implications (1). Newer devices in which the electrode is inserted subdermally over a 3- to 5-day period allow continuous monitoring but are susceptible to drift and may require recalibration. These devices are not approved for therapeutic intervention, and a fingerstick measurement must be taken before administration of insulin (2). Some of the drift seen with these devices may be attributed to their reliance on enzymes (e.g., glucose oxidase) and the well-known tendency of proteins to denature or aggre-gate over time, which may affect their catalytic performance (3–5).

Alternative approaches being actively developed, with potential to provide more reliable long-term continuous monitoring for people with diabetes, use the group of synthetic receptors called phenylboronic acids (6–9). These Lewis acids can bind the cis-1,2- or -1,3-diols of glucose covalently to form 5- or 6-part rings. Unusually, the binding of saccharides is reversible, allowing a direct measurement of glucose, in contrast to enzyme-based methods.

The present study is the 1st time that glucose has been measured in a real physiologic liquid (human blood plasma) using phenylboronic acids. Other phenylboronic acid–based sensor studies have resorted to using simple buffer solutions with selected additives such as sodium chloride or proteins (10–12). This study is also the 1st to test a phenylboronic acid–based sensor for suitable temporal response characteristics that would enable following changes in blood plasma glucose in real time. We used a novel holographic princi-
ple, first developed by Lowe and colleagues \((13, 14)\), wherein glucose is monitored using phenylboronic acids covalently immobilized within a thin-film biocompatible hydrogel, into which a holographic grating has been recorded. In response to analyte-receptor binding and fluctuating glucose concentrations, hydrogel swelling and contraction increase or decrease the spacing between the holographic fringes of the diffraction grating, thereby modulating the color of the light that is diffracted in a manner determined by Bragg’s law. The advantage of this novel detection method over optical techniques such as fluorescence is the long-term stability of the sensor and the ease with which the wavelength may be tuned to suit the application \(\text{see the Data Supplement that accompanies the online version of this article http://www.clinchem.org/content/vol53/issue10}\). Fluorophores, in contrast, often have physical characteristics \(\text{e.g., excitation and emission maxima}\) inappropriate for real applications for which light attenuation, background fluorescence, and photobleaching are possible \((15, 16)\).

The physical and chemical robustness of these holographic sensors allows them to be used continuously for extended periods without the need for recalibration. In addition, these sensors are format-flexible and potentially may be incorporated into strips, catheters, contact lenses, and subdermal implants, thereby providing an attractive alternative to conventional electrochemical enzyme-based methods of glucose monitoring for people afflicted with diabetes.

**Materials and Methods**

The materials and equipment used to make the holograms and the materials for the buffer solutions are listed in Supplemental Data. Human blood plasma \(\text{(single donor and pooled)}\) was purchased from Scipac, Patricell, Cambridge Biosciences, and Valley Biomedical. Samples were supplied with either Na EDTA or K\(_2\) EDTA as the anticoagulant, divided into aliquots, and stored at \(-20^\circ C\) on receipt \((17, 18)\). We used fresh plasma samples for each experiment. In accordance with guidelines, the samples were thawed from frozen through incubation at \(37^\circ C\) in a water bath to prevent cryoprecipitation \((19)\). We measured sample pH by use of a Hanna Instruments pH 213 Microprocessor pH meter or an ELIT 8 channel pH analyzer \((\text{NICO2000 Ltd.) equipped with a Hamilton Biotrode or a Jencons P 16 microelectrode designed for use in small-volume proteinaceous samples. The pH microelectrodes were calibrated with a 2-point calibration using fresh 20-mL sachets of ready-mixed pH buffer \(\text{(Hanna Instruments, NIST traceable) at pH 7.01 and either pH 4.01 or 10.01 as appropriate before measurement. We used peristaltic pumps (Ismatec Reglo digital ISM 834) fitted with Tygon tubing (Ismatec SC0061 and SC0068 set for flow rates of 78.4 \(\mu\text{L/min} \text{ and 175 } \mu\text{L/min, respectively)}\) for glucose-tracking experiments.**

**SENSOR HOLOGRAM PREPARATION**

We prepared 3 mol% \(N,N\text{-methylene bisacrylamide (MBA), 1}\) 12 mol% 3-acrylamidophenylboronic acid \((3\text{-APB})\), 12 mol% \(3\text{-acrylamidopropyl}\text{trimethylammonium chloride (ATMA)}\) holograms on glass microscope slides as described \((20)\) with a few modifications \(\text{see Supplemental Data.}\) The formulation of the polymer hydrogel is expressed in terms of mol%, which reflects the molar amounts of each monomer in the dry thin-film hydrogel. To obtain the hydrogels the appropriate quantities of acrylamide, MBA, 3-APB, and ATMA to give the desired molar ratios are dissolved in a solution of 2\% \(\text{wt/vol)}\) 2,2-dimethoxy-2-phenylacetophenone in DMSO at a ratio of 1:2:21 \(\text{wt/vol)}\) of monomers to solvent. After polymerization the DMSO is removed and the hydrogel is dried down. We assume that there is 100\% conversion of the monomers to polymer.

**GLUCOSE MONITORING IN BLOOD PLASMA SAMPLES**

**Plasma sample preparation and pH control.** Ex vivo plasma becomes more alkaline when exposed to the atmosphere owing to the continual loss of carbon dioxide and its role in the carbonic acid–bicarbonate buffering mechanism \((21)\). To limit the effects of sample pH fluctuations during use and mimic conditions in vivo, we used 2 methods for pH control. The method used to create samples for glucose-tracking experiments required buffering the sample within the physiologic pH range with sodium phosphate, producing a final phosphate concentration of 0.1 mol/L. The buffered solution was then exposed to the atmosphere for 16 h to equilibrate pCO\(_2\) before heating to \(37^\circ C\) and measuring the final pH. The sample was subsequently sterilized by passing through a 0.45-\(\mu\text{m Minisart High Flow syringe filter (Sartorius). In the 2nd method, used for static cell experiments, CO}_2\) was infused directly into the sample using a syringe to lower the sample pH to 7.2, and the diffraction wavelength of the hologram was read when the sample pH attained pH 7.4. Commercially available human plasma is typically supplied within the normal range of glucose concentrations for adults, 4.1 to 5.9 mmol/L \((19)\). To create a panel of samples covering the pathologic range of glucose concentrations, we enriched samples with additional glucose. Samples were allowed to equilibrate for a minimum of 45 min before use, in accordance with ISO guidelines to allow time for mutarotation of glucose \((22)\).

**Hologram glucose interrogation and system-specific sensor calibration.** Sensor holograms were interrogated from the glass substrate side as described \((20)\) with a few modifi-

---

1 Nonstandard abbreviations: MBA, \(N,N\text{-methylene bisacrylamide MBA; 3-APB, 3-acrylamidophenylboronic acid; ATMA, (3-acrylamidopropyl)trimethylammonium chloride.}\)
cations (see Supplemental Data). We calibrated the sensors by comparing sensor diffraction wavelength with sample glucose concentration measured using a YSI 2300 STATplus glucose analyzer. The holographic sensor directly detects analyte molality (moles of analyte per volume of water) of the undiluted sample, and the YSI 2300 measures analyte molarity (moles of analyte per volume of solution) in a diluted sample. For calibration, we used a plasma standard to decrease the systematic error produced through the difference in water content on the YSI 2300–measured glucose concentration, which would occur if an aqueous buffer were used (18, 23).

Static calibration. Each hologram slice was equilibrated in 1.5 mL PBS (10 mmol/L phosphate, 2.7 mmol/L potassium chloride, 137 mmol/L sodium chloride solution, pH 7.40 at 25 °C) to establish a 0 glucose baseline before removal of the PBS and addition of 1.5 mL plasma. Sample pH was manipulated as described above; after the sample attained pH 7.4, the sensor diffraction wavelength was measured and a 60-μL aliquot of plasma was removed from the cuvette for YSI 2300 analysis to establish sample glucose concentration. We added a 60-μL aliquot of 0.1 mol/L glucose-containing plasma. The sample pH was then lowered, and this process was repeated until a glucose concentration >30 mmol/L was recorded on the YSI 2300. The nonlinear calibration data were fitted using the modified single 3-parameter exponential decay function in SigmaPlot 9.

Flow calibration. The sensor cuvette was filled with 1.5 mL buffered plasma, and a peristaltic pump was 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-min intervals, a 60-μL sample of plasma was removed from the cuvette for analysis on YSI 2300, and a 60-μL aliquot of 0.1 mol/L 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 procedure was repeated until a 17 mmol/L 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. We performed sample pH manipulation as stated above and recorded the sensor wavelength when pH 7.4 was attained. Simultaneously, we measured the sample glucose concentration on the YSI 2300. We used the plot equation function in SigmaPlot 9 to interpolate sample concentration from sensor response.

Holographic-based continuous glucose sensing in plasma. Glucose tracking was achieved through a fluidic system using 2 peristaltic pumps (see Supplemental Data). Both peristaltic pumps were primed and initialized 10 min 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 (44 mmol/L) stock plasma at a rate of 78.4 μL/min into a stirred plasma reservoir containing 15 mL plasma. The reservoir had an initial theoretical rate of change of 0.23 mmol/L/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 min with a plasma sample containing a 1.39 mmol/L lower glucose concentration, aiming for a rate of change of 0.17 mmol/L/min. Throughout the procedure, 40-μL plasma samples were collected every 2 to 5 min from the cuvette for glucose measurement by YSI 2300, and the sensor diffraction wavelength was recorded. After a complete cycle of glucose concentrations, the sensor was washed into PBS for overnight storage. We collected daily glucose excursions in plasma over a 3-day period without further calibration. Glucose rate error grids were constructed following methods used in the original report and a subsequent appraisal (24, 25). We collected and paired sensor readings for glucose concentration and their reference blood glucose counterparts every 2 to 5 min over the course of the experiment. Glucose rate of change was calculated using 3- and 15-min time intervals interpolated from the fitted data. The glucose concentration vs time curves for both the sensor and the reference were fitted using the 5-parameter Weibull regression analysis in SigmaPlot 9.0. The sensor and reference plasma glucose rate of change was calculated as the change in glucose concentration of the sample divided by the elapsed time (mmol/L/min).

Results

In Fig. 1, we show the color of a 3 mol% MBA, 12 mol% 3-APB, 12 mol% ATMA hologram in human blood plasma at pH 7.4 and 37 °C as a function of the glucose concentration. The results of using holographic glucose sensors with this hydrogel formulation to measure 46 samples with different glucose concentrations at pH 7.4 and 37 °C are plotted in Fig. 2 against the true reference values obtained using a YSI 2300 (26). The data pairs are plotted as a Clarke error grid, which separates the results into zones reflecting their clinical significance (27). Of 46 measurements, 42 (91.3%) are located in zone A (i.e., accurate), whereas 4 of 46 measurements (8.7%) are located in zone B (i.e., benign outcome). Table 1 shows the results of an experiment performed after collection of the Clarke error grid data to assess measurement imprecision. The measurements performed under flow at pH 7.4 and 37 °C while varying the blood plasma glucose concentration at approximately 0.17 to 0.28 mmol/L/min.
(i.e., at approximately the maximum rate of change of blood glucose in vivo) are represented in the form of rate error grids in Fig. 3 (24, 25). The number of observations made at each rate is listed in Table 2. Using data interpolation and a 15-min time interval, 34 of 34 readings (100%) are located in zone $A_R$ (i.e., accurate) with 0 of 34 measurements located in the $uB_R$ (i.e., upper benign) or $lB_R$ (i.e., lower benign) zones.

Fig. 4 shows how the peak diffraction wavelength of the hologram changes with glucose concentration for different pH values of human blood plasma.

**Discussion**

Based on the results of a previous study looking at sugar selectivity, we chose to prepare thin-film holographic sensors composed of 73 mol% acrylamide, 3 mol% MBA, 12 mol% 3-APB, and 12 mol% ATMA (20) (see Supplemental Data). The 3-APB Lewis acid receptor endows the hydrogel with saccharide responsivity, and ATMA is included at the specified concentration because of its ability to modify the sugar response of the sensor and increase its selectivity for glucose over other saccharides possessing cis-1,2- or -1,3-diols, including the main potential interferent for phenylboronic acids, fructose.

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 ($\lambda_{\text{max}} = 2nd \sin \theta$). The end result is a characteristic spectral reflection peak, the wavelength of which can be correlated with glucose concentration. Using the recording conditions, we obtained holograms that replayed in the red to yellow region of the visible spectrum (Fig. 1). With this sensor, the presence of glucose specifically causes the diffraction peak to blue-shift, indicative of hydrogel contraction of the film perpendicular to the fringe planes. Previously, we proposed that the contraction is caused by cross-linking of 2 boronic acid receptors with favorable stereochemistry by glucose to give a bis-boronate-glucose complex (20).

We generated calibration plots by incrementally increasing the blood plasma glucose concentration and recording the color of the test holograms spectrophotometrically. The glucose concentration of 46 unknowns was then determined from their respective hologram colors using the fit parameters from the calibration curves. Fig. 2 displays the data in the form of a Clarke error grid. The data pairs are scattered about the 45-degree line, demonstrating a good correlation between the test and reference measurement methods. Because 91.3% of the points fall in zone $A$, the test values deviate by $<20\%$ from the true glucose value or both the test value and the true glucose value are $<3.9$ mmol/L. Because 8.7% of the data points fall in zone $B$, the deviation from the true glucose value is $>20\%$, but clinically no treatment is needed or, if treated, the result is

<table>
<thead>
<tr>
<th>Rate</th>
<th>15 min</th>
<th>3 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–0.06 mmol/L/min</td>
<td>7</td>
<td>35</td>
</tr>
<tr>
<td>0.06–0.11 mmol/L/min</td>
<td>8</td>
<td>47</td>
</tr>
<tr>
<td>0.11–0.17 mmol/L/min</td>
<td>12</td>
<td>54</td>
</tr>
<tr>
<td>0.17–0.22 mmol/L/min</td>
<td>6</td>
<td>34</td>
</tr>
</tbody>
</table>

**Table 1. Results of an experiment to assess the measurement precision (reproducibility and repeatability) associated with the experimental test system.**

Fig. 1. The color of a 3 mol% MBA, 12 mol% 3-APB, 12 mol% ATMA hologram in human blood plasma at pH 7.4 and 37 °C as a function of glucose concentration.

Fig. 2. The measurement of 46 human blood plasma samples with different glucose concentrations obtained using a 3 mol% MBA, 12 mol% 3-APB, 12 mol% ATMA hologram at pH 7.4 and 37 °C. The results are plotted against the true values obtained using an accepted reference method (YSI glucose analyzer) and presented in the form of a Clarke error grid.
benign. No points fall in zones C through E, which correspond to errors that are potentially dangerous.

The %CV for the test sensor compares favorably with the YSI reference technique (Table 1). It is anticipated that improvements to the experimental protocols, for instance used to account for the significant pH variation of the samples ex vivo, will lead to further measurement accuracy and precision (see Supplemental Data). One benefit of the holograms is that they behave extremely similarly (see Supplemental Data). In the future, this finding raises the possibility of being able to mass-manufacture materials that do not require calibration before use.

To see if the sensors would be suitable for real-time continuous glucose monitoring, particularly useful for patients with type 1 (insulin-dependent) diabetes, we conducted flow experiments to see if the sensor could track changes in glucose occurring up to the maximum rate of change in vivo. The rate of change of blood glucose and its distribution for the diabetic population has been characterized by Dunn et al. (28). Rates in excess of 0.17 mmol/L/min (upward and downward) are observed in 1% of observations, whereas the majority of the time (approximately 37% of observations), blood glucose changes in the body at 0.06 mmol/L/min. The number of observations at each different rate is listed in Table 2. Fig. 3 shows the rate error grids obtained from the data for 3 consecutive up and down concentration-time curves (see Supplemental Data) assuming fixed time intervals of 3 and 15 min for analysis. The plot reflects the difference in rate between the test sensor and corresponding reference measurements and indicates how well the holographic sensor is able to follow changes in the direction and tempo of plasma glucose. Using a 15-min interval, good accuracy is obtained with 34 readings (100%) located in zone AR (i.e., accurate readings) and 0 of 34 measurements in the upper and lower benign zones (uBR and lBR). Better rate accuracy is obtained if longer time intervals between consecutive measurements are used (see Supplemental Data). Part of this relates to the larger measurement error associated with smaller glucose changes obtained over shorter time intervals.

A common concern regarding hydrogels based on phenylboronic acids is their relatively slow swelling...
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 (29). There has been much discussion about the physical form of the glucose binding to the phenylboronic acid (30). Glucose undergoes mutarotation between its different anomers. It is postulated that the glucose α-anomers bind faster than the β-anomers, but the overall response is poor because the relative abundance of α-anomers is low and interconversion between the different diastereoisomers is slow. In this study, we show for the 1st time that the response characteristics of phenylboronic acid holographic glucose sensors is perfectly adequate for real applications for which continuous monitoring is a benefit, for instance in type 1 diabetes or to facilitate intensive insulin therapy in critical care settings (31).

The ability of these holographic sensors to monitor in real time over long periods without hysteresis makes them ideally suited to continuous glucose monitoring. A benefit of these hydrogels for such applications is their inherent simplicity and biocompatibility (32–34), which is expected to lead to more reliable monitoring. Temperature effects on the signal will be minimized if used in vivo, because the temperature remains essentially constant between 36.1 and 37.8 °C (35). Our hydrogels showed limited temperature sensitivity. Tests measurements made at 36 and 38 °C of human blood plasma containing glucose at 30, 8, and 4 mmol/L all fell within zone A of the Clarke error grid. The plasmas used in this work were at normal physiologic ionic strength (275–300 mOsmol/kg) and are expected to contain normal levels of potentially interfering substances. However, zone A results were also obtained with samples whose ionic strength was adjusted to critical or panic alert levels reflecting a life-threatening situation (250–325 mOsmol/kg) (18).

Another possible cause of signal drift and measurement error is pH. Again the pH of whole blood/plasma is usually tightly controlled between 7.35 and 7.45. However, conditions such as diabetic ketoacidosis and ketoalkalosis may cause pH to fall outside the normal range (36). To investigate how changes in blood/plasma pH affect the glucose measurement, samples of human blood plasma at 6 different glucose concentrations were incubated at 37 °C, and the pH was allowed to drift from 7.2 to 7.7 (Fig. 4). From this data, it is very simple to build a calibration plot for every glucose concentration at every pH value and use this result to obtain an accurate glucose measurement. Many boronic acids have pKₐ values above physiologic pH (37). Therefore, as the pH of the surrounding medium increases towards the pKₐ of the phenylboronic acid in the hydrogel (3-APB ~8.2) (38), the phenylboronic acids switch to their tetrahedral boronate form. This configuration is thought to be better able to bind the vicinal diols of glucose on steric grounds. Thus, the degree of bis-boronate-glucose cross-linking at a given glucose concentration increases with pH, leading to increased hydrogel contraction. Strategies to minimize the pH dependence of phenylboronic acids include modification of the aromatic ring with electron-withdrawing groups so that the tetrahedral form is predominant at physiologic pH and the incorporation of electron donors adjacent to the boron to force the boron into its tetrahedral boronate form (39). The latter method is not as effective, because the electron donors can become protonated or deprotonated if the pH of the surrounding medium changes greatly. Future work will examine the behavior of 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 that is insensitive to changes in pH (40). It is anticipated that the use of these receptors will remove the need outside the normal physiologic pH range for a separate pH measurement as a control.

In conclusion, phenylboronic acid–based sensors can accurately measure glucose in real biological fluids and track changes in concentration occurring at rates mimicking those in vivo. Future work will examine the behavior of these sensors in the presence of different therapeutic substances under physiologic conditions corresponding to different disease states using phenylboronic acid receptors with reduced pH sensitivity.

Grant/funding support: We thank the U.K. Department of Trade and Industry for funding via the Knowledge Transfer Partnership scheme for Dr. Michael Thatcher, a University of Bath KTP Associate with Smart Holograms Ltd. Financial disclosures: None declared.

Acknowledgments: We are grateful to Prof. Chris Lowe (Institute of Biotechnology, University of Cambridge) and Drs. Tony James and Steve Bull (Department of Chemistry, University of Bath) for useful discussions.

References

2. DEXCOM™ STS™ Continuous glucose monitoring system - DEXCOM 2006;L59551, Rev. 04.
5. Akhtar MS, Ahmad A, Bhakuni V. Divalent cation induced changes in structural properties of the dimeric enzyme glucose oxidase: dual effect of dimer stabilization and dissociation with loss of cooperative interactions in enzyme monomer. Biochemistry 2002;41:7142–9.