



Glucose Sensing Using Hydrogel By Incorporating Phenylboronic Acid Groups

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Abstract. Background: Research of glucose detection is important in diabetes management and biosensor development. Taking into account the large amount of water retention, the biocompatibility of hydrogels makes them ideal candidates for glucose detection. The PBA groups, when grafted to hydrogel matrices, improve the glucose-related response of the hydrogels significantly. **Objective:** To create a novel PBA (phenylboronic acid) holographic glucose sensor, MAAM-co-4VPBA, for uninterrupted monitoring of blood glucose levels, which demonstrates the first successful glucose measurement in whole blood using PBA sensors. **Methods:** Methylacrylamide (MAAm) was copolymerized with 4-vinyl phenyl boronic acid (4-VPBA) by means of free radical polymerization employing 1,6-hexanedioldiacrylate (HDODA) as a crosslinker and the photoinitiator 1-hydroxycyclohexyl phenyl ketone. The responsive glucose behavior of the resulting polymer was characterized in terms of swelling dynamics, ex vivo flow tests, and error grid analysis mechanisms. **Results:** The MAAM-co-4VPBA polymer exhibits reversible glucose binding via PBA-diol interactions that allow complexation at different pH and concentration levels. Modulation of the responsive elements of the microcapsules by hydrophobic PBA and hydrophilic MAAM units yields maximum swelling and shrinking dynamics at 37 trail degrees. The sensor successfully detected glucose in opaque biological fluids, blood plasma, without any interference from antibiotics or other therapeutics or endogenous compounds. Ex vivo tests showed real-time glucose monitoring without hysteresis. Most importantly, this work is the first to report the use of PBA-based sensors in whole blood for measuring glucose.

Conclusion: The MAAM-co-4VPBA holographic sensor possesses outstanding features such as accurate records and strong resistance to chemicals and slow response to detection, confirming its effectiveness in continuous glucose monitoring. Moreover, the ability to operate in the real world enables the aid in clinical diagnosis of diabetes.

Keywords: Phenylboronic Acid, Glucose, Maam –CO-VPB, Maam, 4-Vinyl Phenylboronic Acid, 1,6-Hexanediol Diacrylate.

1. INTRODUCTION

Glucose detection is a hot topic in biosensor devices. People suffering from diabetes are always on the lookout for more reliable means of monitoring their glucose levels. New methods are being researched, and non-invasive techniques for detecting glucose were created as safe substitutes that enable continuous monitoring. However, these innovative techniques are still not perfect, calling for the proposal of new methods [1-3]. Glucose sensing is a widely researched field, particularly with the advent of biosensors. Phenyl boronic acid has been proven reliable in continuous, non-invasive glucose monitoring, to the extent that some devices are even branded as accurate [4, 5]. With the help of hydrogels, glucose can be detected and monitored, dramatically improving the endeavors of diabetes patients. Hydrogels utilize omniscapability, which is why they have been guaranteed to prove reliable towards biocompatible devices. What's remarkable is that when glucose-sensing hydrogels are

incorporated with phenyl boronic acid groups, glucose detection is amplified multi-fold [6, 7]. Non-enzymatic phenylboronic acid sensors are unique. They can form robust micelles that bind with the diol forms of glucose, responsible for linking together the gluconeogenesis building blocks [8, 9]. New electrodes are being developed and researched that are less fragile than the enzyme electrodes. Instead of one glucose molecule, they aim to capture glucose polymers [10, 11]. In addition to PBA sensors, different configurations of glucose biosensors are available. Glucose sensors utilizing phenylboronic acid on a basic scale have been developed, which is the reason why there are still many researchers up till today who do not consider the idea handy [12].

We have demonstrated for the first time that a sensor based on phenylboronic acid can function in blood plasma, which differentiates it from traditional systems that utilize simplified buffers with specific proteins and sodium chloride. This research looks into how the sensor can be used for real-time blood glucose measurement, showing data collected in the controlled setting of blood plasma. The results of the study ascertained that real-time glucose change rates can be measured by the sensor, maintaining comparability with in vivo conditions. In addition, we study the effect of a wide range of other substances, including analgesics, antibiotics, anticoagulants, antidiabetic agents, blood preservatives, and some metabolites, on the accuracy of the measurements. We employ an innovative approach of glucose sensing developed by Lowe and associates [13, 14]. This technique involves embedding phenylboronic acid receptors in a hydrogel that has a holographic grating. The hydrogel shrinks or swells when glucose binds to the receptors, altering the distance between the holographic grating hinges. Consequently, the wavelength of the diffracted light is altered, as described by Bragg's law. Thus, glucose is detected optically. Different from fluorescence optical sensors, this one is more stable, tunable, and easier to apply in other fields. In addition, the phenylboronic acid receptors make it possible for these sensors to be older without constant recalibration, which makes these sensors more attractive, comprehensive substitutes for the enzyme-based electrochemical glucose monitors, particularly for people suffering from diabetes mellitus.

2. MATERIALS AND APPROACHES

Chemical And Instrumental

1. Materials

Glucose, Methylacrylamide (MAAm) Purity (99.99%), M.Wt (85.11), was purchased from HIMEDIA. 4-vinyl phenylboronic acid (4-VPB) Purity (99.99%), M.wt (147.97), was purchased from HIMEDIA. 1,6-Hexanediol diacrylate (HDODA) Purity (99.99%), M.Wt (226.27) was purchased from ALDRICH. And 1-hydroxy cyclohexyl phenol ketone (photo initiator PI184) Purity (99.99%), M.wt (73.9), was purchased from HIMEDIA.

2. Instrument

Fourier transform infrared (FTIR) spectroscopy Spectrophotometer: used to measuring absorbance at 450 nm, the mixer centrifuge, the USpicy downlight, CRESCENT USND-1801 18W LED, 4 x 9W UV radiation, works very effectively and safely, and it's simple to clean and control with the use of the timer for 120 seconds or without timing for an indefinite amount of time, and using it for the monomers' photopolymerization after mixing them in the gel tube, incubation, and Spector.

3. Quantitative Determination of Glucose:

Synthesis of copolymer (MAAm-VPBA) (working reagent)

When crosslinking agent 1,6-hexanedioldiacrylate (HDODA) and photoinitiator PI184 (1-hydroxy cyclohexyl phenyl ketone) are present, the hydrogel monomers methyl acrylic amide (MAAm) and vinylphenylboronic acid (VPBA) are polymerized via free-radical polymerization. Agitate the mixture for half an hour at room temperature with nitrogen (N₂) in a dark environment. The slurry was dried under UV light at 365 nm for 40 minutes after being placed into polypropylene molds. After demolding, the copolymer was cleaned by soaking it overnight in 50% ethanol at 50°C to eliminate the unreacted monomers and photoinitiator. The polymer was then submerged in distilled water at 50 °C for 12 hours to remove the remaining ethanol. HDODA was present in 0.625 weight percent and PI184 in 0.4 weight percent.

Samples

Plasma or serum and whole blood that is hemolysis-free should be extracted from the clot as soon as possible.

Assay condition

At temperature 37°C, wavelength 340 nm, the cuvette cell has a light path of one centimeter, using distilled water to reset the device to zero, and pipette into a cuvette.

Table 1. Procedure

Parameters	Standard	Blank	Sample
WR (ml)	1,0	1,0	1,0
Standard ml	10	-	-
Sample	-	-	10

After combining, incubate the samples at room temperature for 25 minutes or at 37°C for 10 minutes, then the absorbance (A) of the samples and the standard should be recorded against the blank.

Calculation:

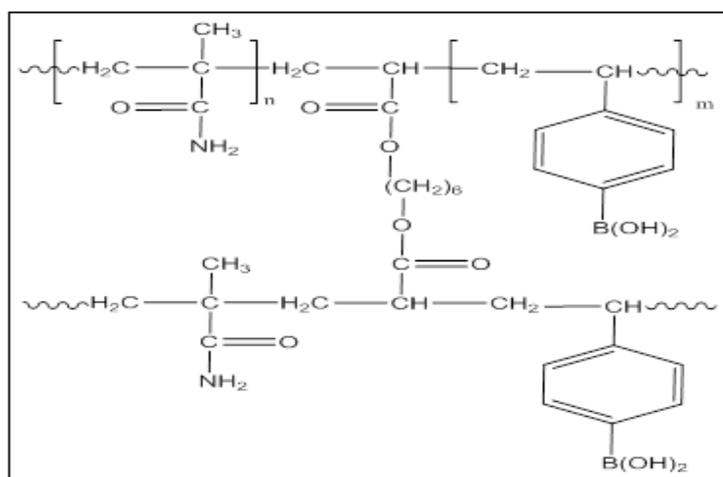
$$\text{glucose (mg/dl) in the sample} = \frac{(\text{A) sample} * 100 (\text{standard conc.})}{(\text{A) standard}}$$

Conversion factor: mg/dl * 0.0555 = mmol/L.

3. RESULT AND DISCUSSION

Synthesis and characterization of polymer (MAAm-VPBA) (working reagent)

The work reagent was made by combining methyl acrylic amide (MAAm) and vinylphenylboronic acid (VPBA), then by free radical polymerization they were polymerized in the presence of 1,6-hexanedioldimethacrylate (HDODA) as a crosslinking agent, and the PI184 (1-hydroxy cyclohexyl phenyl ketone) as photoinitiator for 30 minutes and at room temperature in the presence of nitrogen N₂.



Scheme 1. Synthesis of cp-polymer (MAAm-VPBA) (working reagent)

FTIR spectrum of MAAm-CO-VPBA

The infrared spectrum, as depicted in Fig.1, displayed many peaks, the most notable of which is a broad peak in the region (3200-3400) cm^{-1} , which denotes the overlap between the absorption bands of both (OH) and (NH) bonds. The characteristic band at frequency 1670 cm^{-1} refers to the stretch vibration of the bond (C=O) belonging to the amide group, while the bond peak (C=C) of aromatics appears at frequency (2890-2980 cm^{-1}) and corresponds to the stretching vibrations of the aliphatic C-H bonds in the polymer structure. (1548 cm^{-1}). The stretching vibrations of the (C-H) bonds of the polymer's CH_3 groups are represented by the distinct peaks in the range (1375-1450 cm^{-1}), the absorption peaks at frequencies (1100-1120 cm^{-1}), the stretching vibrations of the C-B bond, and the absorption peaks for the B-O bond in the range (1220-1240 cm^{-1}), respectively [15].

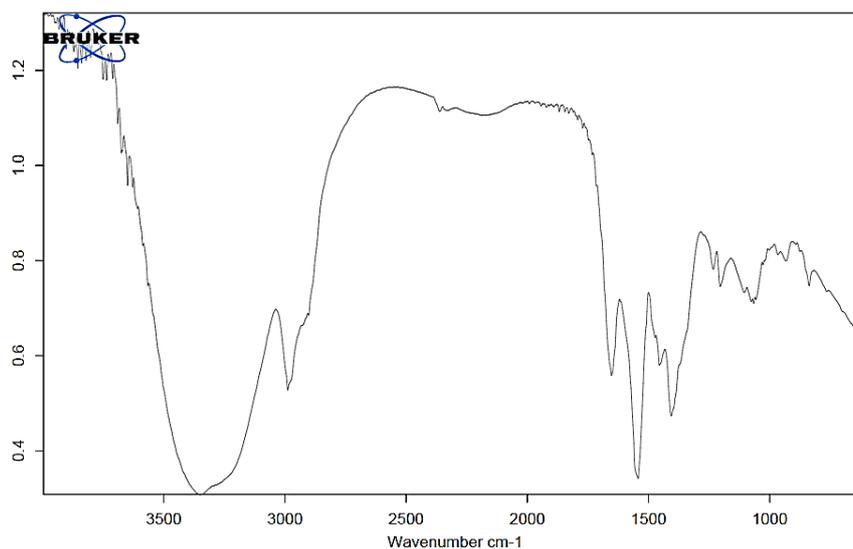


Figure 1. FTIR spectra of (MAAm-CO- VPBA)

Effect of Ph on Glucose Sensitivity

The contraction and swelling behavior of the MAAm-co-VPBA hydrogel is responsive to glucose concentration and pH relative to its pK_a , therefore, when the pH levels are lower than pK_a , the hydrogel increasingly swells with increasing glucose concentration as a result of reversible crosslink formation between one molecule of glucose and two moieties from PBA on different chains of polymer. In contrast, when the pH is greater than the pK_a , the hydrogel contracts, and the glucose concentration increases. This is explained by the interaction of PBA and an amine group in MAAm, which lowers the pK_a value of the hydrogel. This means that the MAAm-co-VPBA hydrogel can undertake contraction on increasing glucose concentrations at a much lower pH than normal (7.4). Particularly, the hydrogel did not exhibit

contraction when placed in fructose solutions, highlighting the specificity of the crosslinking mechanism in the gel towards glucose in MAAm-co-VPBA gels. In Fig. 2, it has been demonstrated that the glucose responsiveness of the polymer was analyzed for an extensive pH range. The polymer was placed in glucose solutions of different concentrations, and its absorbance was measured under various acidic conditions (pH=2, 4, 6). It was found that the glucose sensitivity was greater than 6, indicating that this hydrogel system could be used more generally at pH > 2 for glucose detection.

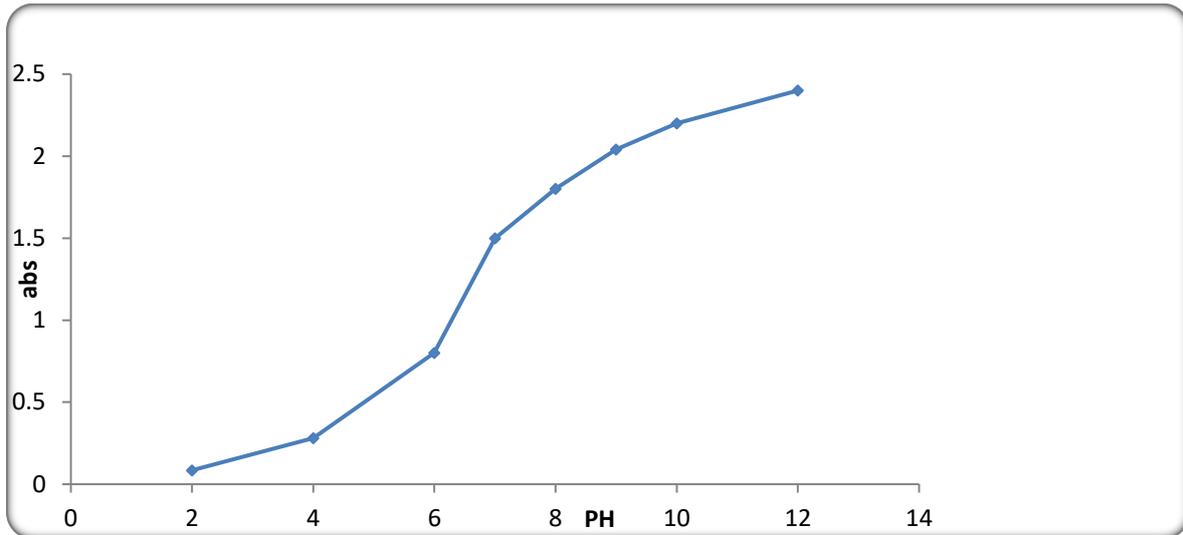


Figure 2. Show sensitization to glucose at physiological

52.7% of the study's patients had at least one problem, and 57.8% had poor glycemic control. Poor glycemic control was independently predicted by the following factors: age, income, hypertension, duration of disease, and hypercholesterolemia. Only age, education, and the length of the condition, however, independently predicted problems. The middle and older age groups had better glycemic control than the younger age group, where other factors did not affect the association. Similar to this, several research studies indicated that older age groups had improved glycemic control. On the other hand, complications were worse as people got older. This was still important even after taking into account a number of confounders. Senior diabetics are more likely to die, develop problems, and require hospitalization [16-18]. The results of our study, in particular, were in agreement with those of these investigations, i.e., although the older age group had better glycemic control, they experienced the highest percentage of issues. where findings revealed that senior diabetes individuals experienced more problems while having better glucose control. This may highlight the challenge it is to controlling diabetes in elderly persons, resulting in more thorough risk profiles for glycemic

control. Although older diabetic patients showed better glucose control, functional ability, and comorbidities may explain the greater complications and advise a wider geriatric examination.

The Design and Fabrication Strategy for The Polymer Glucose-Responsive

The sensor DMA-co-VPB can reversibly react and attach itself to glucose, as shown in Figure 3 [19]. In unsaturated form, PBA derivatives are capable of going through a phase change to a saturated form, both of which are soluble in water. Glucose can also react with both forms of PBA freely. Specifically, the unsaturated form is prone to hydrolysis and unstable; however, the covalently bonded PBA to glucose is only stable in a charged state. At roughly 32°C, the actuator DMA-co-VPB turns into an easily recognizable thermoresponsive material with a volume phase transition VPTT. This means that the material can change between swollen and shrunken states depending on the temperature, and the state change temperature for DMA-co-VPB is 37°C. To reach the greatest rate of glucose-sensing-dependent PBA swelling and shrinking changes at 37 degrees Celsius, we include hydrophobic DMA and PBA. The structure of the proposed glucose-responsive polymer is shown in Figure 3. The glucose-responsive polymer is stated to shrink at 37°C in the buffered solution close to the pKa of the DMA-co-VPB part (pKa = 8.6), where the PBA is assumed to exist in the free and protonated states [19, 20].

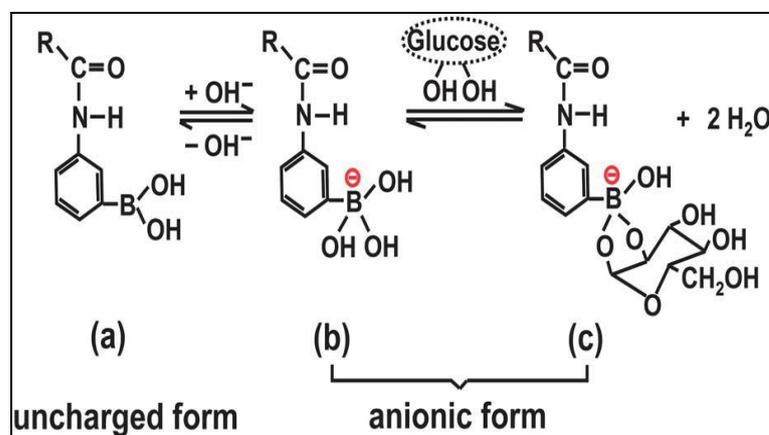


Figure 3. Illustration of the phenylboronic acid-glucose complex in aqueous solution.

When the concentration of glucose increases, the PBA charged form in the hydrogel shell can form a stable complex with glucose by reversible covalent bonding. The complexing of the phenyl borate-containing moieties of PBA consumes ionic PBA forms, therefore disrupting the dissociation equilibrium of PBA and transforming the associated phenyl borate group into more hydrophilic, uncharged, nonionic PBA. This results in the movement of the polymer's VPTT

to an increase in the temperature and Donnan potential, leading to exhibiting higher swelling in the microcapsules at a glucose concentration of 37°C. The changes in VPTT of DMA-co-VPB copolymer may represent the phenomenon of glucose-responsive microgel with significantly enhanced responsiveness in terms of glucose-triggered swelling during copolymer phase separation [21, 22]. The PBA-glucose complex cleaves in response to an increase in glucose concentration, the donning of capsule walls also results in a decrease in glucose concentration, which leads to the microcapsule undergoing glucose-induced shrinking. Given certain physiological conditions, polymers with glucose-responsive swelling and shrinking properties can greatly facilitate self-regulated glucose delivery systems for cancer and diabetes treatment [23].

The basic method by which PBA reacts to sugar molecules is depicted in Fig. 4 [24]. In an alkaline environment, the Lewis acid boron, which has empty sp^2 orbitals, reacts with the Lewis base hydroxide ion to form a negatively charged ion. The Condensation of the complex tetrahedral boronate negative charge with planar cis-diols sugar molecules stabilizes the charged form. The condensation of Sugar with the PBA uncharged trigonal acid form can also occur, but not as frequently. These negatively charged PBA groups produce an osmotic swelling force that causes the expanded hydrogel when they are bonded to it. Fig. 4 shows that either high concentration of sugar or high pH, or both, can promote edema [24].

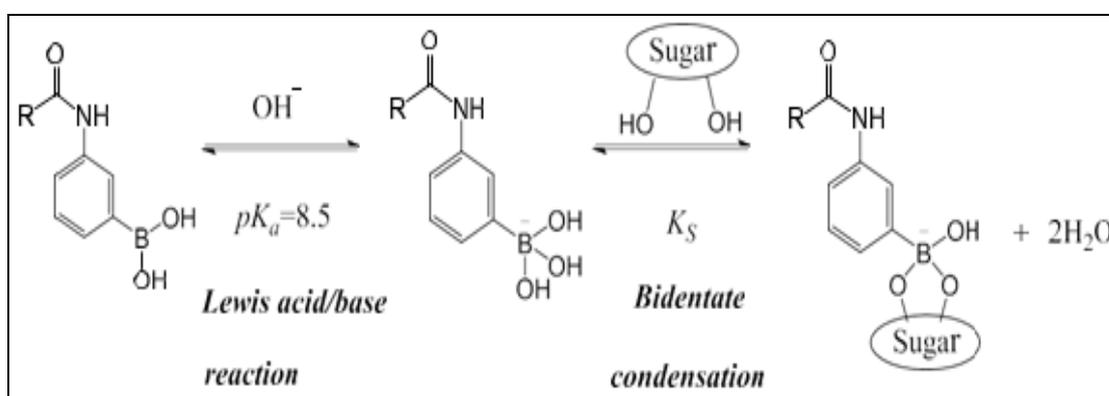


Figure 4. Shows the promotion of concentration of sugar and pH on edema.

In addition to being sensitive to the target analyte, a crucial feature of any sensor is its specificity. PBA binds additional sugars besides glucose, as was already mentioned. The significance of this effect depends on the relative abundances of each potential interfering species and the PBA's affinity for glucose about their respective abundances. However, glucose is different from other common sugar types in an interesting way. As seen in Fig. 4, a single glucose molecule can build a bisbidentate bridge when two sets of planar diols interact with

PBA moieties on different polymer chains. The hydrogel shrinks as a result of the formation of such (reversible) bridges, which effectively increases crosslinking. The majority of other sugars, including fructose, can only attach to one PBA moiety.

The first generally discussed reporter event in the field of polymers containing BA is the swelling of a network of cross-linked polymers. Such polymeric systems develop a glucose-dependent permeability that enables their usage in an autonomous insulin administration system. When glucose is present or the pH increases due to the BA-equilibrium shifting to the charged side, these polymeric systems swell. As the glucose is absorbed by the cells and withdrawn from the bloodstream, the reversibility of such a device would also reduce the drug's release, preventing hypoglycemia shock.

The synthesis and characterization of hydrogel systems for applications of glucose sensing, as well as mathematical description of hydrogel swelling, were reported in this work. The main glucose detection techniques still in use today are enzymes, especially glucose oxidase. Alternative approaches, such as using phenylboronic acid receptors, have been proposed. These receptors allow for exceptional direct continuous measurement due to their ability to bind glucose through covalent bonds. We studied the capability of Phenylboronic-based sensors in blood and blood plasma, as well as the effects of various potential interferents on measurement accuracy. We also showed that the sensor continuously measures the rates of glucose concentration changes during vigorous exercise. We performed static in vitro blood and blood plasma glucose measurements with holographic sensors containing MAAm-co-VPB. These same sensors were used in other in vitro tests conducted under flow conditions.

The optical sensor's ability to track blood or plasma glucose concentration was not affected by the turbidity of the liquid. Error grid analysis showed that measurement accuracy was not affected by the most commonly used antibiotics, diabetic drugs, analgesics, or other endogenous compounds. Ex vivo flow experiments confirmed that real-time measurements are possible without any time delays or hysteresis in concentration change. Blood glucose levels were first measured by phenylboronic acid sensors. Because the holographic sensors don't require calibration or adjustment, they are the most promising choices for continuous blood glucose monitoring from a thermodynamic and kinetic standpoint. For this study, human plasma was selected to test the sensor's capabilities in a real-life physiological environment with contaminants. Plasma was easier to work with than blood because, if stored correctly, it can last a long time. The plasmas used for this study were anticipated to contain typical background amounts of both exogenous and endogenous chemicals. Concerning actual biological fluids, we have demonstrated that phenylboronic acid sensors' capacity to measure

glucose, and to monitor the change in concentration of glucose at comparable rates to that occurring in vivo. In this work, the glucose-sensitive holograms fabricated and tested may also be capable of providing no calibration required measurements as well as more reliable continuous monitoring.

Statistical Analysis

SPSS software 22 was used for statistical analysis. The data was analyzed based on the T-test application used to compare the difference between the two groups. The data are presented as mean ±S.D. The cutoff for significance statistically was p 0.05. (HbA1c: Glycated hemoglobin; *indicates substantial variations from the control).

Table 2. Comparison of Age and HbA1c in the two study groups

<i>Parameters</i>	<i>Control group Mean ±SD</i>	<i>Patients group Mean ±SD</i>	<i>P-value</i>
<i>Age</i>	28.321±20.878	21.826±18.581	0.322
<i>HbA1c</i>	4.996%±0.74	9.201%±1.916	< 0.001*

Data from Table 2. The two groups' age differences were not statistically significant (p > 0.05). but HbA1c levels were. The control group's HbA1c was less than 5.7%, which is considered normal, as shown in the control group, while the diabetic patient group's was 6.5% or higher, indicating diabetes. Hemoglobin A1c is the diagnostic test for diabetes.

Table 3. The values of the blood glucose levels for the healthy control and patient's groups using the usual kit and the laboratory- repaired kit.

<i>Parameters</i>	<i>BS kit (mg/ dl), Mean ±SD</i>	<i>BS preparation kit (mg/ dl), Mean ±SD</i>	<i>P-value</i>
<i>RBS Control group</i>	101.739± 12.604	100.985 ±13.230	0.619
<i>RBS Patients group</i>	229.155± 56.985	233.427±55.771	0.893

(RBS: Random blood sugar, BS kit: Blood sugar kit)

Data from Table 3. Shows there are no significant differences between measuring the level of blood sugar by using the usual kit and the laboratory-prepared kit. More reliable continuous monitoring.

4. CONCLUSIONS

We have shown that polymer sensors based on phenylboronic acid are capable of precisely measuring glucose in actual biological fluids and monitoring concentration changes at rates similar to those observed in vivo. The prepared polymer showed a great response to glucose at the physiological pH, and this was reinforced by the strategies used during the preparation of the various polymers, containing in their structure amino groups or electron withdrawing groups that enabled the high response to glucose under physiological conditions (temperature 37°C, pH 7.4). The data shows there are no significant differences between measuring the level of blood sugar by using the usual kit and the laboratory-prepared kit.

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