

Supplementary Information

Degradation of Hydrogel Beads for the Detection of Serum Bicarbonate Levels for the Diagnosis of Metabolic Alkalosis at the Point of Care

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Table of Contents

Table S-1. Comparison of our hydrogel-based assay with the gold standard methods for detecting bicarbonate.

Figure S-1. Structural components of the POC device.

Table S-2. Demonstration of the reproducibility of the bicarbonate detection assay in PBS.

Figure S-2. Relative blue intensity (RBI) measurements for complete trials **A**, **B**, and **C** in **Table S-2**.

Figure S-3. Temperature dependence of 0.6% calcium alginate and 40% dextran 6k hydrogel bead degradation in PBS.

Figure S-4. Study of 0.6% calcium alginate and 40% dextran 6k hydrogel beads.

Video S-1. Video of working POC device.

Table S-3. The results of three complete trials for the degradation of 0.6% calcium alginate and 40% dextran 6k hydrogel beads in human serum using the POC device.

Materials and Methods

Hydrogel bead morphology, stability, and dye retention study

To develop a diagnostic assay with a long shelf-life, it is necessary for the hydrogel beads to be both stable in water and capable of retaining dye for an extended period of time. Therefore, the stability and dye release properties of the optimized CaAD hydrogel beads made with 0.6% calcium alginate and 40% dextran 6k were studied in water over time. Due to the dye being blue, absorbance values of the outer solution were taken at 595 nm immediately after hydrogel synthesis, after 4 days, and after 8 days, to quantitatively measure dye release over time.

Demonstration of bicarbonate detection with the device

One 2 mL microcentrifuge tube was prepared with 1.49 mL of human serum (Lot # 502036415, Fisher Scientific, Hampton, NH) spiked with 50 mmol/L of sodium bicarbonate. Next, 15 μ L of citric acid in PBS at a concentration of 1 g/mL was added into the tube. 10 CaAD hydrogel beads were then transferred into the tube to start the degradation reaction. The tubes were placed on the POC device at room temperature. The battery pack was then turned on. A video of the working device was taken with an iPhone 12 camera.

Results and Discussion

Comparison of our novel bicarbonate detection assay with the current gold standards

Our hydrogel-based assay was compared with the current gold standard methods for bicarbonate detection, which are arterial blood gas analysis and basic metabolic panel analysis, in **Table S-1**.

Table S-1. Comparison of our hydrogel-based assay with the gold standard methods for detecting bicarbonate.

Method of detection	Arterial Blood Gas Analysis (ABG) ^{1, 2, 3, 4} *Based on MSLAB36 Blood Gas Analyzer	Basic Metabolic Panel (BMP) ^{4, 5} *Based on Piccolo Blood Chemistry Analyzer	Our Hydrogel-Based Assay
Sensitivity	Reportable range for pCO ₂ is 5.0-200.0 mmHg*	Reportable range for tCO ₂ is 5-50 mmol/L**	Sensitive for bicarbonate concentrations 35 mmol/L and above
Need for Trained Personnel	Needed for sample extraction, sample preparation, handling laboratory equipment, interpreting results, and performing Allen's test	Needed for sample extraction, sample preparation, handling laboratory equipment, and interpreting results	Needed for sample extraction and preparation
Equipment	Equipment for blood extraction and serum purification; arterial blood gas analyzer instrument	Equipment for blood extraction and serum purification; enzymes for bicarbonate detection; chemistry analyzer instrument	Equipment for blood extraction and serum purification; miniature and portable POC device

Time-to-Result	Laboratory personnel would get results in 10 to 15 min but delivery of results to patient might take >1 day	Laboratory personnel would get result in 14 min but delivery of results to patient might take >1 day	10 min - 1 h in a mobile clinic
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*The serum bicarbonate concentration can be calculated with the pH and the partial pressure of CO₂ (pCO₂) using the Henderson-Hasselbalch equation

**The serum bicarbonate concentration comprises about 95% of the content of total CO₂ (tCO₂) detected with the respective enzymatic reaction⁶

Structural components of the POC device

The components that make up the POC device are shown in Figure S-1.

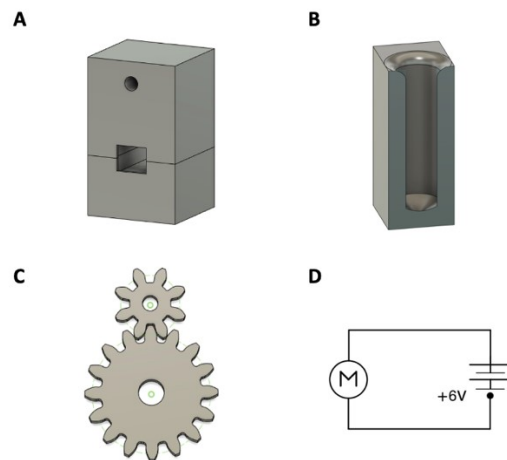


Figure S-1. Structural components of the POC device. (A) DC motor housing. (B) Tube holder. (C) Gears. (D) Electrical circuit.

Demonstration of the reproducibility of the bicarbonate detection assay in PBS

The methods describing the assay concerning the degradation of 0.6% calcium alginate and 40% dextran 6k hydrogel beads were repeated to demonstrate the reproducibility of this technique for the detection of sodium bicarbonate in PBS. The results were very consistent as shown in **Table S-2**.

Table S-2. The results of 10 complete trials for the degradation of 0.6% calcium alginate and 40% dextran 6k hydrogel beads in PBS.

[Bicarbonate] (mmol/L)	A (min)	B (min)	C (min)	D (min)	E (min)	F (min)	G (min)	H (min)	I (min)	J (min)	AVG (min)	Std dev (min)	Std error (+/-) (min)
50	15	15	16	16	17	17	16	15	15	14	15.60	0.97	0.31
45	23	22	22	26	20	24	21	24	20	22	22.40	1.90	0.60
40	26	29	29	33	35	35	36	39	40	43	34.50	5.38	1.70
35	84	106	113	95	107	125	105	81	101	80	99.70	14.70	4.65

Demonstration of the reproducibility of the bicarbonate detection assay in PBS using RBI analysis

The RBI values for the first three panels of the 10 reproducibility trials are represented in **Figure S-2**.

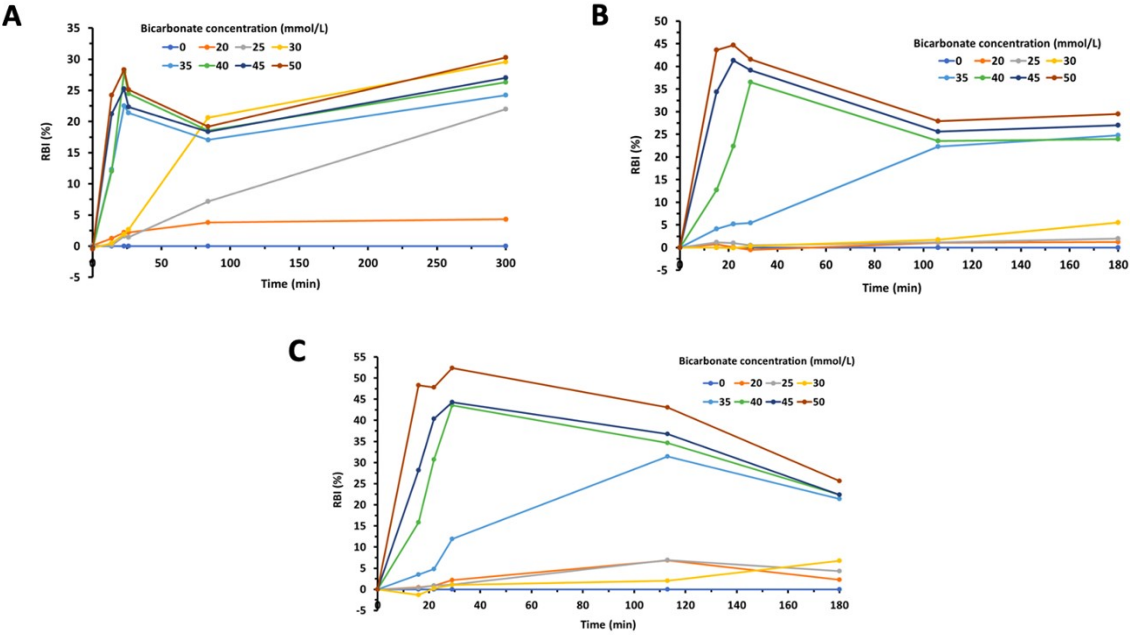


Figure S-2. Relative blue intensity (RBI) measurements for complete trials **A**, **B**, and **C** in **Table S-2**.

Effect of temperature on the degradation kinetics of the hydrogel beads

Degradation of 0.6% calcium alginate and 40% dextran 6k hydrogel beads was investigated at 35°C. The visual results of the trial as well as the RBI values are shown in **Figure S-3**. When comparing these results with the degradation study at room temperature, seen in **Figure 5B**, it is evident that temperature influences the kinetics of the assay. Nevertheless, the degradation trend is still visible, with a clear dependence on bicarbonate concentration.

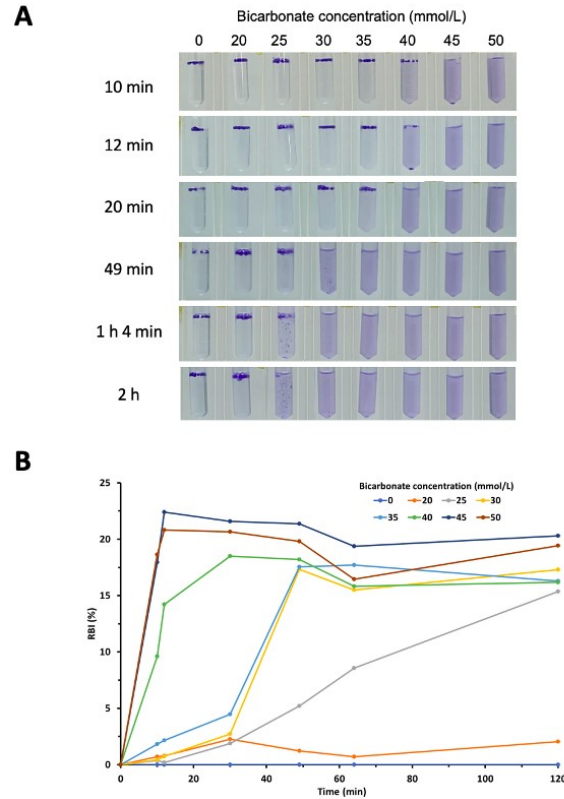


Figure S-3. Temperature dependence of 0.6% calcium alginate and 40% dextran 6k hydrogel bead degradation in PBS. **(A)** Photos and **(B)** RBI values at different time points.

Hydrogel bead morphology, stability, and dye retention study

0.6% calcium alginate and 40% dextran 6k hydrogel beads showed ideal spherical morphology (**Figure S-4A**), as well as sustained dye retention and stability over an 8-day period (**Figure S-4B**). This was further verified by the very slight increase in absorbance at 595 nm from -0.001 to 0.067 on day 4, followed by another very slight increase to 0.096 on day 8. This gradual increase in absorbance is quite negligible when compared to the absorbance values of hydrogels undergoing bicarbonate induced degradation, observed in **Figure 6B**. Overall, these properties make the hydrogel beads suitable for a diagnostic assay with a long-shelf life.

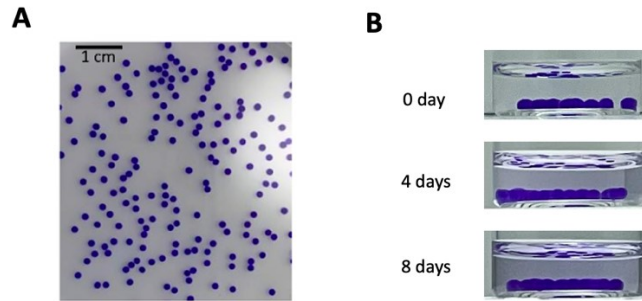


Figure S-4. Study of 0.6% calcium alginate and 40% dextran 6k hydrogel beads. **(A)** Overall morphology. **(B)** Dye release study.

Demonstration of bicarbonate detection with the device

The CaAD hydrogel beads in 50 mmol/L sodium bicarbonate in human serum fully degraded in 10 min. The video demonstration for the trial is included in **Video S-1**.

Video S-1. Video of the working POC device. A microcentrifuge tube holder is attached to a rotating motor powered by 4 AA batteries within a battery housing.

Demonstration of the reproducibility of the bicarbonate detection assay in human serum

The methods describing the assay concerning the degradation of 0.6% calcium alginate and 40% dextran 6k hydrogel beads were repeated to demonstrate the reproducibility of this technique for the detection of sodium bicarbonate in human serum. The results are shown in **Table S-3**.

Table S-3. The results of three complete trials for the degradation of 0.6% calcium alginate and 40% dextran 6k hydrogel beads in human serum using the POC device.

[Bicarbonate] (mmol/L)	A-2 (min)	B-2 (min)	C-2 (min)	AVG (min)	Std dev (min)	Std error (+/-) (min)
50	10	16	18	14.67	4.16	2.40
40	23	33	39	31.67	8.08	4.67
30	100	120	129	116.33	14.84	8.57

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