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Toward a Quantitative Colorimeter for Point-of-Care Nitrite Detection

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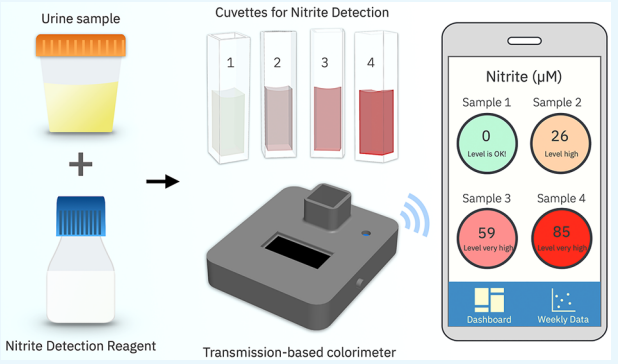
ABSTRACT: This paper reports on a low-cost, quantitative, point-of-care solution for the early detection of nitrite, a common biomarker for urinary tract infections (UTIs). In a healthy individual, nitrite is not found in the urine. However, a subject with a suspected UTI will produce nitrite in their urine since the bacteria present will convert nitrate into nitrite. Traditionally, nitrite is monitored by urinary dipsticks that are either read by eye or using a reflectance spectrophotometer. Both methods provide a semiquantitative positive or negative result at best. In this paper, we described a novel, affordable, portable transmission-based colorimeter for the quantitative measurement of nitrite. A unique permutation of the Griess reaction was optimized for the clinical detection of nitrite in urine and is reported. By using nitrite spiked in a salt buffer, artificial, and human urine samples, the performance of the colorimeter was evaluated against dipsticks read using two commercial dipstick analyzers, Urisys 1100 (Roche Diagnostics) and Clinitek Status+ (Siemens Medical Solutions). The colorimeter was able to detect the clinically relevant range of nitrite from 0.78 to 200 μM in a salt buffer. The detection limit in artificial urine was determined as 1.6 μM, which is ∼16× more sensitive than commercial dipstick reflectance analyzers, enabling the possibility for earlier detection of urinary infections. The colorimeter is assembled using off-the-shelf components (<$80) and controlled by a smartphone application *via* low-energy bluetooth. It has a built-in color correction algorithm and is designed to enable for a turbidity correction in samples containing bacteria or other cellular debris as well. The mobile application can display the nitrite concentration for a single sample or display the results over a period of time. Tracking urinalysis results longitudinally can help identify trends such as increases in nitrite concentrations over an individual's baseline and identify possible infections earlier. While the detection of nitrite was showcased here, this portable analyzer can be expanded to other colorimetric-based chemistries to detect a panel of biomarkers, which can improve the overall sensitivity and specificity of the desired assay.



INTRODUCTION

Typically, dipsticks are used to measure the concentration of different analytes in urine. Dipsticks are disposable, inexpensive to manufacture, easy-to-use, and read visually by eye. However, these readings are subjective and error-prone, and the accuracy is highly dependent on (1) proper sample preparation, (2) correct interpretation of the reference color scale, and (3) precise timing of the readout. Proper sample preparation involves using a dip-and-wipe method of submerging the dipstick into the sample and then carefully wiping the edge of the dipstick along the rim of a cup to remove excess liquid. This ensures that each test pad is exposed to the correct volume of liquid. A test pad that is exposed to too little or too much liquid can introduce errors in the color readout. The different shades of color of the test pad will vary depending on the illumination conditions, causing potential errors when the dipstick is read visually by eye. Individuals who are color-blind would inherently be unable to read and interpret these dipsticks. Moreover, a typical urine test strip has five or more test pads that may need to be read at different times. For example, the glucose test pad needs to be read at 30 s, nitrite and protein need to be read at 60 s, and leukocytes can be read at 120 s after dipping into the liquid. Having several reagent pads with the same readout time and requiring the user to interpret the results simultaneously against a reference scale marked on the bottle may be difficult for an untrained user. Given these challenges of dipstick urinalysis, it is difficult to obtain good accuracy with dipsticks when used in an at-home environment.

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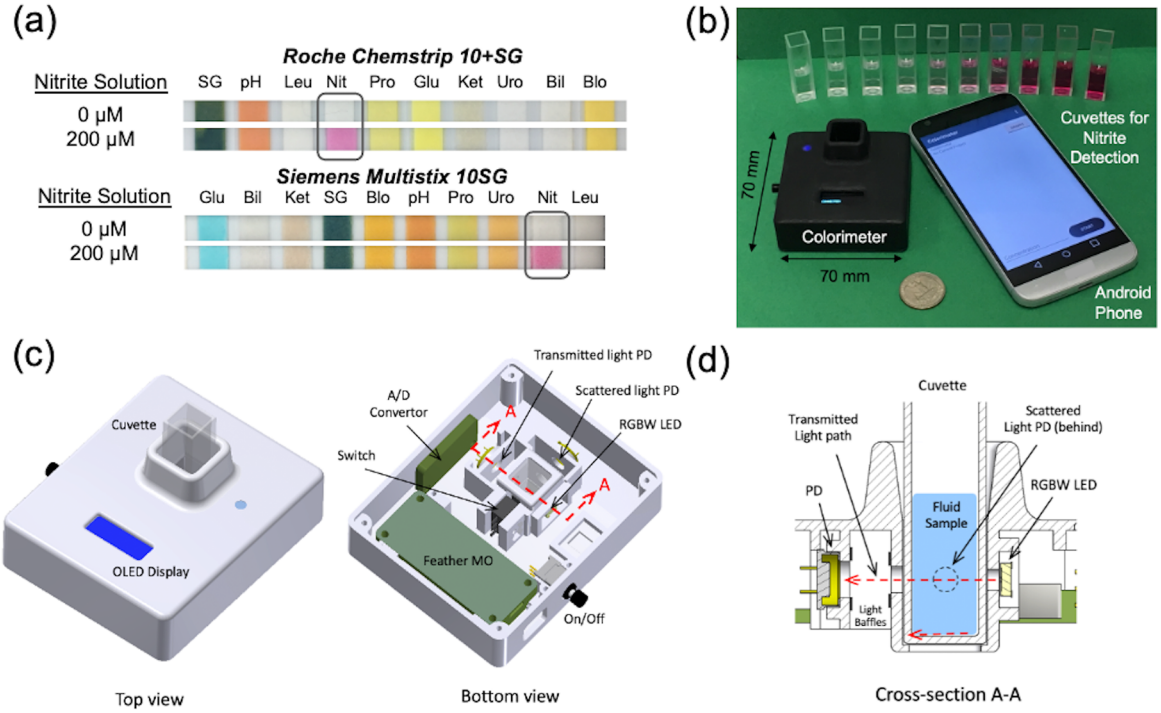

Figure 1. (a) Example of two Roche Chemstrip 10+SG and Siemens Multistix 10SG urine dipsticks dipped in a 5 mM hydrion buffer solution of 0 and 200 μM nitrite at pH 5.4. The dipsticks were read by the Roche Urisys 1100 and Siemens Clinitek Status+ analyzers, respectively. (b) Image of the quantitative, transmission-based colorimeter, Android phone, and cuvettes containing reagents for nitrite detection. (c) Schematic showing the top and bottom views of the colorimeter and its internal components. (d) Cross-section view of A-A shows the placement of the two photodetectors (PD) of the transmitted and scattered light paths.

In order to improve the accuracy of dipsticks for at-home testing, several companies, such as Healthy.io[1] and Scanwell Health,[2] have included the use of a smartphone to take an image of the dipstick where the color is interpreted using built-in algorithms to eliminate user subjectivity. Other researchers have created a device that controls for uniform lighting and uses video captured by a smartphone to interpret the color and results.[3] Other groups have opted to use paper-based microfluidics to control the sample flow and filter out particles to improve detection sensitivity.[4] However, for all these methods, the dipstick readout is still semiquantitative at best.

In the clinic, benchtop-sized reflectance photometers such as the Urisys 1100 from Roche Diagnostics (Indianapolis, IN) and the Clinitek Status+ from Siemens Medical Solutions (Malvern, PA) are used to electro-optically read the colored pads on a dipstick. A user would place the test strip into a sliding tray, and the measurement is done by capturing the reflected light from three LEDs positioned at a fixed angle above the reading zone.[5] Although present day reagent strips and reflectance photometers have reached a high degree of perfection, there still exists a number of intrinsic drawbacks. For example, using reflected light, the background of the test strip can interfere with the lower limits of sensitivity for many reagents. While it is possible to modify the chemical composition of the reagents to overcome this drawback, chemical modification is often expensive and difficult. Moreover, reflectance photometers report only a binary positive or negative readout in the case of nitrite, and a quantized semiquantitative readout for other analytes like protein and creatinine. This prevents other important metrics such as the protein-to-creatinine ratio to be determined and tracked in conditions like chronic kidney disease.

Thus, a low-cost, portable, and cloud-enabled system that can provide a quantitative readout of analytes in urine would have great value for at-home monitoring and tele-health applications. There has been work reported on using capillary electrophoresis to monitor nitrite in urine, but the >20 min sample processing time and washing steps are complicated for an at-home user.[6−8] Several studies have demonstrated microfluidic technologies that could help simplify sample preparation and achieve precise control of sample volume. However, these devices are often nonreusable and would add cost and complexity to the system.[9−11] Other groups have developed low-cost, wireless spectrophotometers to perform more quantitative measurements.[12,13] However, the use of a mini-spectrophotometer in the design can make the device cost-prohibitive for use in an at-home setting.

In this paper, we describe an affordable, quantitative, portable, transmission-based colorimeter that can measure nitrite in a salt buffer and artificial and human urine samples. Nitrite is an important biomarker for the early detection of urinary tract infections. The bacteria such as *Escherichia coli* present in an infection will convert the nitrate into nitrite, which is detectable in urine. In one study, a positive nitrite detection in urine suggests 99% likelihood to indicate a UTI in children of any age.[14] We explored various permutations of the Griess reaction, a well-known scheme for nitrite detection, and optimized a set of reagents for transmission-based measurements. We compared the performance of the colorimeter with dipsticks read on two reflectance-based dipstick analyzers: the Chemstrip 10+SG test strip read using the Urisys 1100 and the Multistix 10SG test strip read using the Clinitek Status+ (Figure 1a). We found that our transmission-based instrument can detect nitrite as low as 1.6 μM, about 16× more sensitive

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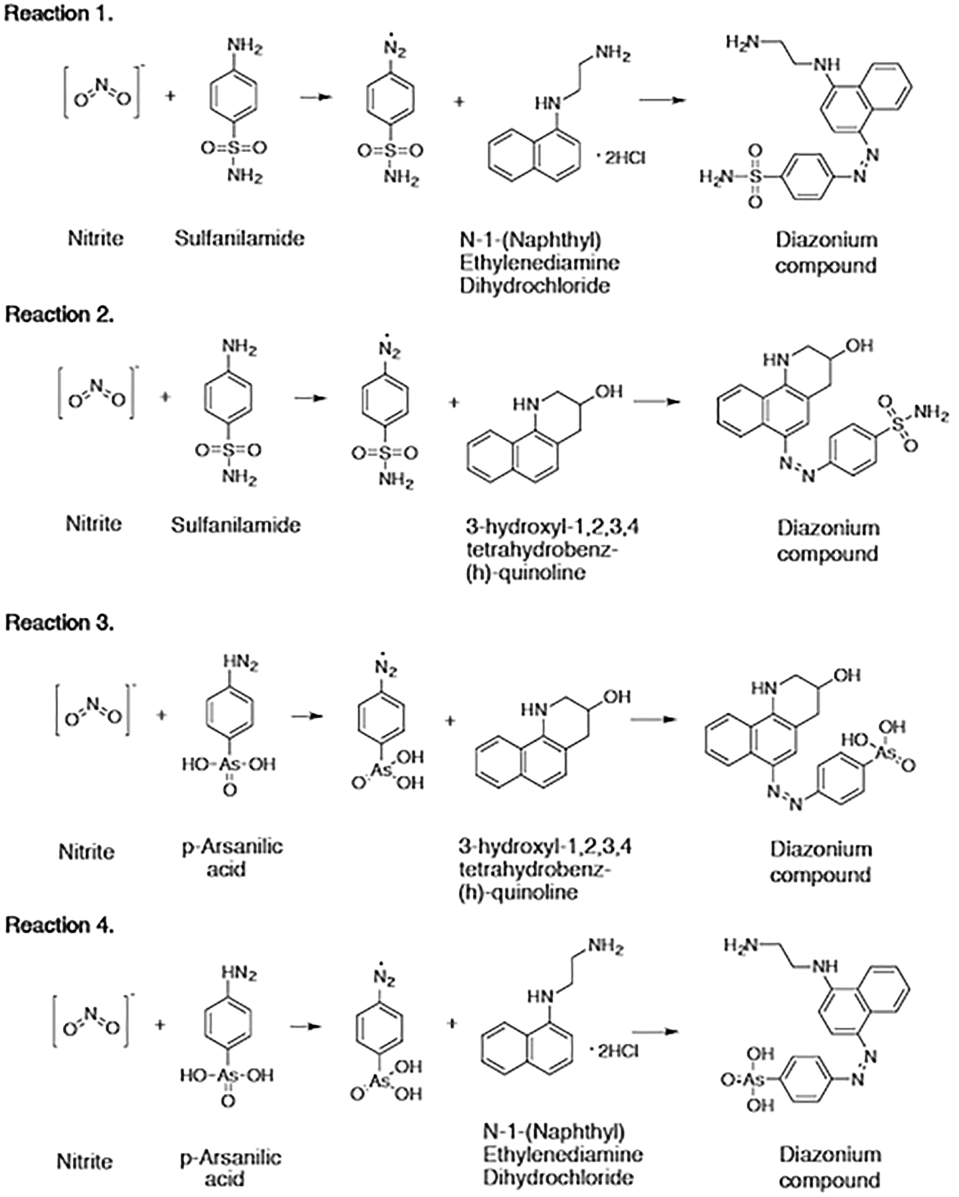
Scheme 1. Schematic of Four Chemical Reactions Based on the Griess Reaction to Detect Nitrite in Solution[a]



[a]Reaction 1 has been established as the European standard for determining nitrite concentrations in drinking water. Reactions 2 and 3 are the nitrite detection chemistries used on the Chemstrip dipstick by Roche and Multistix dipstick by Siemens, respectively. Reaction 4 is a permutation of Reactions 1 and 3.

than the reflectance-based analyzers. A table comparing the performance of our colorimeter and other high performance devices is provided in Table S1. A more sensitive nitrite detection can enable earlier UTI detection and treatment. We also demonstrated the use of a mobile application to report the nitrite concentration for a single event as well as to display readings captured over time. Any increase in nitrite observed over time is also another indicator for the onset of an infection. This device is a first step to bringing an affordable, portable, quantitative urinalysis for at-home patient monitoring.

RESULTS AND DISCUSSION

Colorimeter Device Design. The colorimeter is a hand-held device housed in a 70 × 70 × 21.5 mm^3 3D-printed casing (Figure 1b). Samples are measured using 10 mm path length poly(methyl methacrylate) (PMMA) cuvettes, which have an optimum transmission in the visible spectral range from 340 to 800 nm. The colorimeter was designed to operate in ambient conditions. Top and bottom views of the colorimeter and the placement of its internal components are shown in Figure 1c, and an electronic schematic of all the components and connections is provided in Figure S1. In the sample slot, there is a pair of multiwavelength light-emitted diodes (LED) and a photodetector placed on opposite sides for transmission measurement. The LED is located 8.6 mm from the inside bottom of the cuvette, and a minimum sample volume of 0.8 mL is needed to generate a reproducible transmission reading from a given sample (Figure S2). A second photodetector is placed at 90° to the LED to capture the scattered light that is used for turbidity measurements. A surface-mount red−green−blue−white (RGBW) neopixel LED from Adafruit is used as the light source and was selected for its small form factor and

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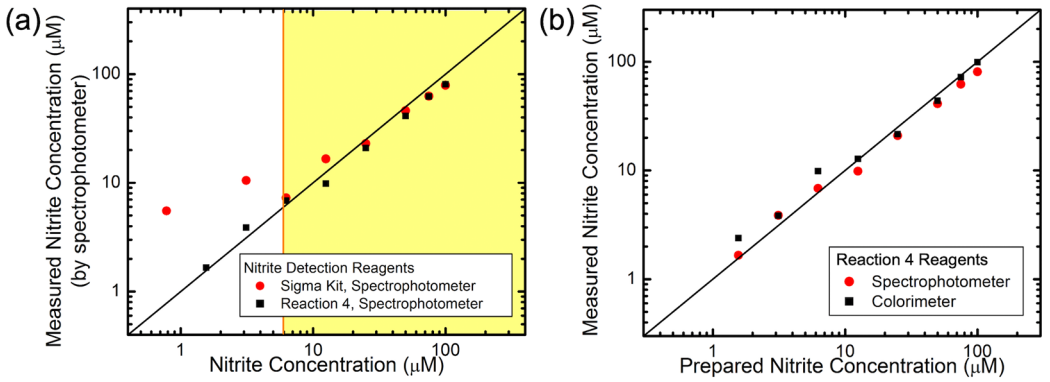

Figure 2. (a) Comparison of the measured nitrite concentration using detection reagents from the Sigma nitrite detection kit (red circles) and the reagents described in Reaction 4 of this work (black squares). The yellow box depicts the suitable nitrite detection range of the Sigma kit. (b) Comparison of the measured versus the prepared nitrite concentrations using reagents described in Reaction 4 on a SpectraMax i3x spectrophotometer (red circles) and a colorimeter (black squares).

digital control. The LED emitting colors had the following peak wavelengths: red (620−625 nm), green (522−525 nm), and blue (465−467 nm). The white wavelength is a quasi-white light generated from the mixture of the RGB wavelengths.[15] The LED is mounted outside of the cuvette holder with a small aperture to expose the LED pixels. The photodetector is installed behind a baffle that is located 10 mm from the aperture to eliminate interference from stray light. Figure 1d provides a cross-sectional view of the transmitted and scattered light paths. An Arduino controller board with low energy bluetooth capability, such as the Adafruit Feather M0 Bluefruit Low Energy board, was used to control the operation of the LEDs and the photodetectors. It was also used to communicate with an Android smartphone *via* an application developed for data collection, analysis, and storage. The Android application can display the concentration of nitrite for a single event or a set of readings over time.

Selection of Nitrite Detection Reagents. Four chemical reactions based on the Griess reaction were identified and evaluated for thte detection of nitrite in a 5 mM hydrion buffer (Scheme 1). The Griess reaction consists of an aniline derivative and a coupling reagent in an acidic solution. The most common arrangement uses sulfanilamide with *N*-(1-naphthyl)ethylenediamine dihydrochloride (NED) (Reaction 1) and has been established as the European standard for measuring nitrite concentration in drinking water (BS EN ISO13395).[16,17] When a sample containing nitrite is added, the nitrite ions react with sulfanilamide in a Griess diazotization reaction to form a diazonium salt. This salt then reacts with NED to form a reddish-pink azo dye that can be quantified using a spectrophotometer to determine the nitrite concentration. Some common aniline derivatives including *p*-arsanilic acid, nitroaniline, and *p*-aminoacetophenone and other coupling reagents such as 3-hydroxyl-1,2,3,4 tetrahydrobenzo-(*h*)-quinoline (THBQ) can be used to change the sensitivity and solubility of the azo dye formation in solution. Reactions 2 and 3 are the chemistries used on the Roche Chemstrip and Siemens Multistix urine dipsticks, respectively.[18−20] Reaction 4 is a permutation of Reactions 1 and 3. While the Griess reaction is well-known for detection of nitrite in solution, we are not aware of studies that characterize and optimize the reaction for use in transmission measurements of urinary nitrite in a clinically relevant range.

Reactions 1−4 were characterized for reproducibility, pH independence, limit of detection, and stability in a 5 mM hydrion salt buffer containing varying known amounts of nitrite. All four reactions were reproducible across 10 readings, and the same response was achieved in solutions of pH 4.3 and pH 7.9. Reactions 1 and 4 had a detection limit of 0.78 μM, which is 8× more sensitive than Reactions 2 and 3. Using THBQ, we noticed a brownish-yellow background coloration even at the lowest concentrations of nitrite (Figure S3a,b). Although the colorimeter has a built-in color correction algorithm, we opted to further evaluate Reactions 1 and 4 which use NED, since the samples remained colorless even at the lower nitrite concentrations. One explanation is that NED is more polar than THBQ as a coupling reagent, hence it will form a more soluble dye in the acidic aqueous medium. It was observed that the solution mixture of Reaction 1 turned pink over time, perhaps due to some impurities in the sulfanilamide powder. Since the solution mixture of Reaction 4 containing *p*-arsanilic acid and NED remained colorless over time, it was chosen and used as the primary nitrite detection scheme for subsequent experiments (Figure S3c).

The detection reagents from Reaction 4 was validated against a nitrite detection kit from Sigma (Cat No: 23479) using a SpectraMax i3x benchtop spectrophotometer. Samples were prepared containing nitrite ranging from 0.78 to 100 μM in a 5 mM hydrion salt buffer at pH 7.0. The Sigma kit has a suitable nitrite detection range between 6.25 and 100 μM. The detection reagents in Reaction 4 were well-correlated with the kit when read using the spectrophotometer (Figure 2a). Interestingly, the Griess reagents from the kit had a narrower dynamic range of nitrite detection compared with the reagents in Reaction 4 as seen by the standard curves in Figure S4a. In the linear range of the Sigma kit, the spectrophotometer reading of the kit is similar to the measurements from the colorimeter using reagents in Reaction 4 (Figure S4b).

Next, measurements on the colorimeter and the spectrophotometer were compared using samples with known nitrite concentrations in a 5 mM hydrion salt buffer at pH 7.0. Both the colorimeter and spectrophotometer detected nitrite between 3.125 to 100 μM and were well-correlated (Figure 2b). This suggests the colorimeter could be used for quantitative measurements, and coupled with its lightweight and portable form-factor, the colorimeter could be translated for at-home use.

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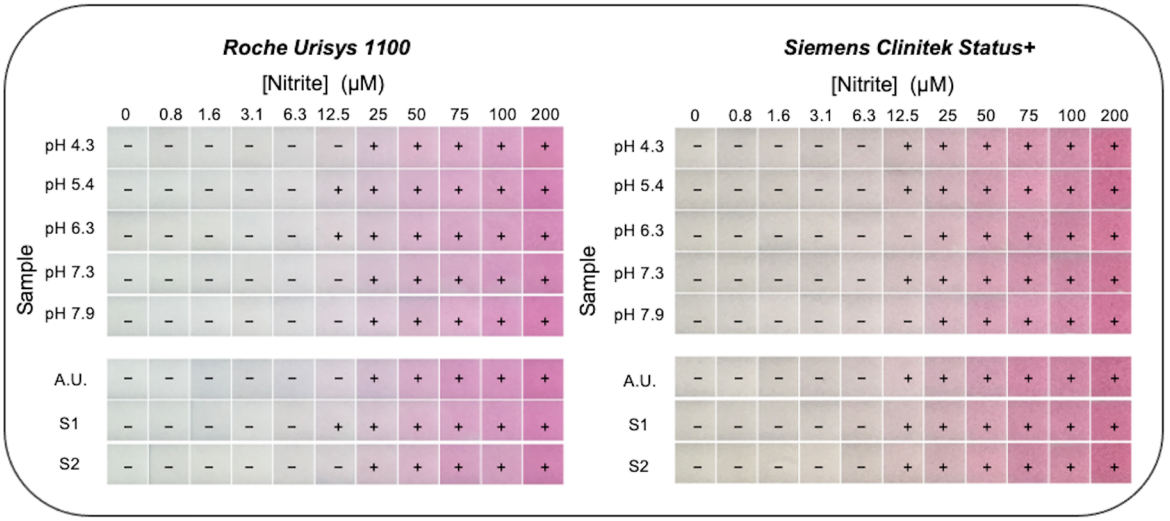

Figure 3. Array of nitrite pads stitched from cropped images of independent Chemstrip 10+SG and Multistix 10SG dipsticks dipped in buffers with varying concentrations of nitrite and pH. The dipsticks were read by a Roche Urisys 1100 and a Siemens Clinitek Status+ analyzer. Minus and plus signs on each pad indicate a negative or positive readout from the two analyzers.

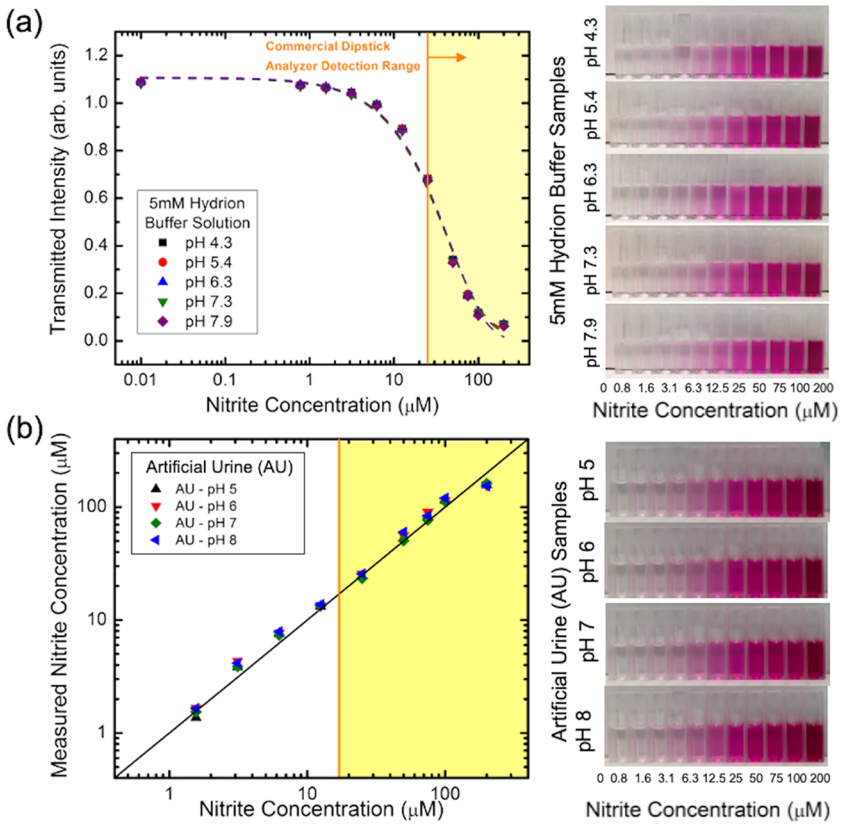

Figure 4. (a) Transmitted intensity was measured using the colorimeter as a function of nitrite concentration for nitrite samples prepared in 5 mM hydrion buffer solution with pH ranging from 4.3 to 7.9. (b) Nitrite concentrations determined from colorimeter measurements taken in artificial urine (AU) (pH 5−8) spiked with varying concentrations of nitrite. The yellow box indicates the detectable sensing range for the dipsticks read using the Siemens and Roche reflectance photometers. Cuvettes containing nitrite detection reagents mixed with nitrite samples are shown.

Nitrite Detection in 5 mM Hydrion Salt Buffer. Serial dilution was used to prepare nitrite samples between 0 and 200 μM in a 5 mM hydrion salt buffer at pH 4.3, 5.4, 6.3, 7.3, and 7.9. The samples were spiked into the PMMA cuvettes containing the nitrite detection reagents. Each reaction occurs very rapidly with a minimal decrease in signal after 60 s. In this study, measurements were taken using the colorimeter after 10 min to ensure each reaction achieved steady-state as shown by the reaction kinetics data in Figure S5. Figure 4a shows the performance of the colorimeter measuring these samples with a nitrite detection limit of 0.8 μM in the salt buffer. Since the azo-dye product formed from the Griess reaction causes the solution to turn pink, the maximum absorption peaks occur at the green wavelengths. Thus, the transmitted light intensity

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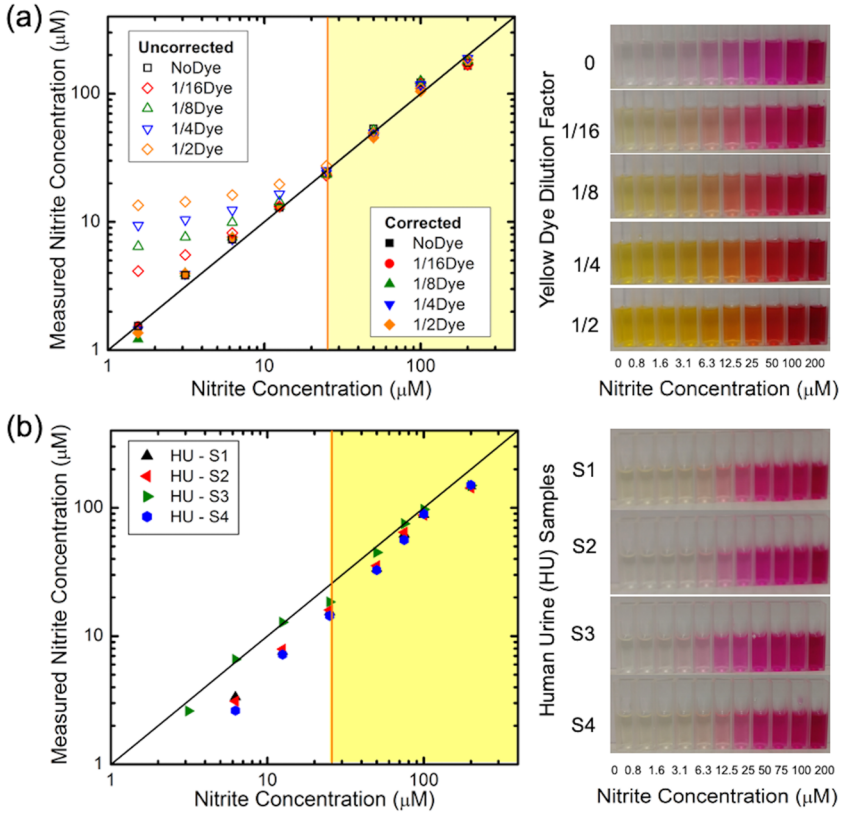

Figure 5. Nitrite concentrations calculated from colorimeter measurements of varying concentrations of nitrite spiked into (a) sequential dilutions of artificial urine (AU) containing a yellow dye additive, with and without color correction, and (b) human urine (HU) samples with the color correction algorithm applied.

from the green channel was used to determine the nitrite concentration, since it was most sensitive to any variations in concentration (Figure S6).

The nitrite samples were also read using the Chemstrip and Multistix dipsticks and their respective reflectance photometers, a Urisys 1100 and a Clinitek Status+. The nitrite reagent area on a urine dipstick will turn from white to pink in the presence of nitrite. This can happen if an individual has a urinary tract infection (UTI), where *Escherichia coli* or other bacteria reduce the nitrate in urine to nitrite.[14,21,22] Since nitrite is not present in healthy urine, any degree of pink coloration would be interpreted as a positive result.

Both the Roche and Siemens dipsticks were used to measure nitrite in the same samples prepared for the colorimeter. While a few samples containing 12.5 μM nitrite were reported as positive by the analyzer at different pH values, it was found that a sample must contain a minimum of 25 μM of nitrite to generate a positive response in a reproducible manner (Figure 3). In comparison, the colorimeter was able to detect nitrite as low as 0.8 μM in a salt buffer, equating to a ~32× improvement in sensitivity.

Nitrite Detection in Artificial Urine. Similarly, nitrite samples were prepared in artificial urine (AU) purchased from Pickering Laboratories. Ready-to use artificial urine solution was chosen since it closely resembled human urine. The formulation contained a nontoxic perservative to avoid bacteria growth and has a pH of 6.5. A new calibration curve was generated with samples containing known concentrations of nitrite in artificial urine at pH 7.0 (Figure S7).

$$y = \frac{0.9888}{1 + (x/32.5921)^{1.6783}} - 0.0059 \quad (1)$$

Equation 1 is the logistic equation used to fit the data, where x is the measured transmitted intensity value from the colorimeter and y is the calculated nitrite concentration present in the sample. For a series of samples spiked with varying amounts of nitrite, the nitrite amount was calculated using the calibration curve. This measured value was reported as a function of the actual nitrite concentration spiked in the sample. The 45° diagonal line is a reference line that shows perfect equality between the measured nitrite concentration versus the prepared nitrite concentration (Figure 4b). Since all the values from the colorimeter lie closely on the 45° diagonal line, the readings from the colorimeter can be used to determine the nitrite concentration in unknown samples with good accuracy.

While the dipstick analyzers provide a binary negative or positive determination of nitrite in a given sample starting at 25 μM, the colorimeter can quantitatively determine exact values of nitrite in samples from 0.8 μM in a salt buffer and 1.6 μM in artificial urine up to 200 μM in clinically relevant pH values from 5−8.

Color Correction Algorithm. Urine can vary from a light, pale straw color for a healthy individual to a dark, cloudy appearance in someone who is dehydrated or sick. Urine can also appear pinkish, red, or even blue depending on the food or medication taken. The color of the urine will impact the transmission measurement and in turn affect the accuracy of nitrite detection. Thus, it was necessary to develop an

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algorithm to remove the color effects from the nitrite measurements.

The nitrite measurement and color correction were performed using the colorimeter. Before the insertion of any sample cuvettes, the on and off intensity for red (R), green (G), blue (B), and white (W) lights are measured by flashing the R, G, B, and W LED pixels on and off and taking the reading of a photo diode. Those readings are denoted as $I_{(cal,i),on}$ for the on intensity of RGBW and $I_{(cal,i),off}$ for the off intensity, where i denotes for R,G,B, and W. Next, a sample cuvette with no preloaded reagents is inserted into the colorimeter, and the transmission intensity of RGBW light is measured through the sample. Those readings are denoted as $I_{(cal,(i)color}$ for light on intensity and $I_{(cal,(i)color,off}$ for light off intensity. Finally, the sample is added to a cuvette containing nitrite detection reagents, and the transmitted intensities for RGBW light are measured again. Those measurements are denoted as $I_{(t,(i)nitrite}$ and $I_{(cal,(i)nitrite,off}$ for on and off transmission intensities. The color of the urine can be expressed as a 16-bit R,G,B value and displayed on the mobile application using eq 2:

$$I_i = \frac{[I_{(cal,i),color} - I_{(cal,i),color,off}]}{[I_{(cal,i),on} - I_{(cal,i),off}]} \times 256 \quad (2)$$

A color correction calculation is applied to determine the corrected amount of nitrite present using eq 3:

$$I_i = \frac{[I_{(t,i),nitrite} - I_{(cal,i),nitrite,off}]}{[I_{(cal,i),color} - I_{(cal,i),color,off}]} \quad (3)$$

where i denotes the red (R), green (G), blue (B), and white (W) colors of the LED illumination (I), and t stands for transmitted light.

To demonstrate the color effects on the detection of nitrite, we added a water-soluble yellowish-brown dye into 10 mM phosphate buffer (pH 6.0) and prepared solutions at 1/2, 1/4, 1/8, and 1/16 dilution. Nitrite was spiked into the diluted dye solutions starting at 200 μM and was serially diluted to zero. A sample without any nitrite added was included as a control. Figure 5a is a plot of the measured nitrite concentration versus the known nitrite concentration in these prepared dye solutions before and after the color correction. It is expected that the 1/2 diluted solution will appear the darkest with the least light transmitted due to its color, while the 1/16 diluted solution will appear the lightest and have the greatest light transmitted. Color correction reduced the error in nitrite detection by as much as 15 μM, which is significant for earlier UTI detection. Without color correction, a sample with a nitrite concentration of 1.6 μM will be mistaken as 12.5 μM in the 1/2 diluted solution.

The performance of the colorimeter and the two dipstick analyzers to detect nitrite spiked in four freshly collected human urine (HU) is shown in Figure 5b. In a normal healthy urine sample, there should be zero nitrite present. Applying the color correction algorithm to four human urine samples spiked with varying concentrations of nitrite showed improved correlation between the measured and known nitrite concentrations in the sample. Three of the four human samples have nitrite values that were slightly under-measured, suggesting potential interference from the sample matrix. It was subsequently found that the presence of urobilinogen can reduce the measured transmitted intensity (data not shown), which will be investigated further.

Besides color, the turbidity of the sample can also affect the accuracy of the colorimeter reading. A urine sample can appear turbid if there are bacteria cells or other debris present. These particles can scatter the incoming light and reduce the amount of light reaching the in-line photodetector. To correct for the turbidity in a given sample, the scattered light is measured using a second photodetector placed at 90° to the in-line photodetector. If a sample is more turbid, the higher amount of scattered light will be captured by this second photodetector. Stabilized formazin turbidity standard sets from Hach were used to generate a calibration curve between the turbidity measurement from the colorimeter and the optical density of the same samples measured using a NanoDrop spectrophotometer (ThermoFisher Scientific). The plot of the optical density versus scattering intensity was used to convert the measurement to a physical turbidity value (Figure S8), and this is displayed on the mobile phone application. For certain applications where the urine sample may appear cloudy, perhaps due to an infection, the colorimeter will be able to determine the level of cloudiness. Additional studies with urine samples spiked with bacteria or cellular debris are needed to develop and validate a turbidity correction algorithm that can further improve the accuracy and sensitivity of the colorimeter for nitrite detection.

Detection Reagent Stability. In order for the assay to be practically used, the nitrite detection reagents should be stable at room temperature or a refrigerated environment for a prolonged period of time. Initial stability studies were conducted by preparing and storing nitrite detection reagents at room temperature, 4 °C, and −20 °C and then testing these reagents with samples containing various concentrations of nitrite in a 5 mM hydrion salt buffer between 0 and 200 μM over a 25 day period. The nitrite readings remained stable for at least 25 days, regardless of storage temperature (Figure S9), suggesting that the nitrite assay can be suitable for at-home or remote use. Further work will investigate the ability to lyophilize the nitrite detection reagents and determine the stability of the dried formulation.

Data Recording and Monitoring. Nitrite values are displayed on the Android mobile application after each measurement. The data are stored locally on the mobile phone, and the results can be displayed as a function of time. This tracking of nitrite values is important to identify any trends such as increases in nitrite concentrations over an individual's baseline, since this can be used as another indication for an infection. The application is programmed to highlight when nitrite levels are normal and display a warning message if the nitrite levels are high (Figure S10). In the next iteration of the colorimeter, the goal is migrate the data analytics and the storage of the data values onto a HIPAA compliant cloud-based server, which would allow for the measurement data to be easily accessible by clinicians or other members of a patient's healthcare team.

CONCLUSIONS

We have demonstrated the first-step toward building an affordable, portable colorimeter for the quantitative measurement of urinary nitrite for at-home monitoring. The colorimeter is built from off-the-shelf components (<$80) with a 3D-printable case. The device pairs *via* bluetooth to an Android phone that can store and report single measurements as well as a time-series of stored results. The Griess reaction used to detect nitrite on traditional dipsticks was modified and

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optimized for use in a transmission-based environment. The colorimeter can detect nitrite as low as 0.8 μM in a salt buffer and 1.6 μM in artificial urine in a quantitative manner. Compared with commercial dipstick reflectance photometers, the ∼16× improvement in sensitivity for nitrite in artificial urine may be useful in detecting and treating urinary tract infections earlier. The nitrite detection reagents were stable for at least 25 days at room temperature, 4 °C, and −20 °C. Next steps include connecting the mobile application to a cloud-based server to perform its data analytics and storage as well as multiplexing the colorimeter to include additional biomarkers such as leukocytes, which are present in the case of an infection. A multiplex panel of biomarkers will improve the sensitivity and specificity of the overall assay and the confidence it has to predict a given disease.

MATERIALS AND METHODS

Chemicals and Materials. Sulfanilamide, *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED), *p*-arsanilic acid, hydrochloric acid (37%), and sodium nitrite were of analytical reagent grade and purchased from Sigma-Aldrich (St. Louis, MO). The Sigma nitrite detection kit (Catalog No. 23479) was also purchased from Sigma-Aldrich. Hydrion buffer chem-velopes at various pH values from 4.0 to 8.0 were purchased from MicroEssential Laboratories (Brooklyn, NY). Artificial urine (Cat. No. 1700-0600) was purchased from Pickering Laboratories (Mountain View, CA). Formazin beads were from the Hach Company (Loveland, CO).

Preparation of Reagents for Nitrite Detection. Using the Griess reaction, which consists of combining an aniline derivative and a coupling reagent in an acidic solution, four arrangements were tested for nitrite detection using the colorimeter (Scheme 1).[23] Reaction 1 was prepared by adding 0.25 g of sulfanilamide to 24.75 of mL distilled water followed by 0.25 mL of hydrochloric acid. The mixture was well-mixed before 0.025 g of *N*-(1-naphthyl)-ethylenediamine dihydrochloride (NED) was added. Reaction 2 was prepared by adding 0.05 g of sulfanilamide to 25 mL of methanol followed by 0.8 g of citric acid and 0.0425 g of 3-hydroxyl-1,2,3,4-tetrahydrobenzo-(*h*)-quinoline (THBQ). Reaction 3 was prepared by adding 0.0625 g of *p*-arsanilic acid to 25 mL of methanol followed by 0.8 g of citric acid and 0.0425 g of THBQ. Lastly, Reaction 4 was prepared by adding 0.0325 g of *p*-arsanilic acid to 24.75 mL of distilled water followed by 0.25 mL of hydrochloric acid (37%). The mixture was well-mixed before 0.0025 g of NED was added. Varying amounts of sodium nitrite were spiked into the hydrion salt buffer, artificial, and human urine samples for testing.

Colorimeter Measurement. To prepare a sample for measurement, the colorimeter is first paired with an Android phone *via* bluetooth. By using the software application, an initial calibration measurement is performed with no cuvette in the sample slot to correct for any possible variations in the LED intensities. Next, a cuvette with no detection reagents but only the sample added is inserted and the color and turbidity of the sample are measured and recorded. Then, 1 mL of sample is added to the cuvettes containing 1 mL of detection reagent and measured. For time-based measurements, the reaction is measured using a colorimeter every 10 s for 10 min. For end-point measurements, a series of 10 measurements are recorded and averaged at 10 min after the reaction has reached a steady state. The software application calculates the nitrite concentration based on the photodetector reading and built-in calibration curve and color interference correction algorithm. The turbidity measurements can in the future be used to correct for any interference from the bacterial or cellular components in the urine sample. The results are digitally displayed on the phone screen and saved as a .csv file locally on the device for downstream analytics.

Dipstick Measurement. Two commercial dipsticks, Roche Chemstrip 10+SG and Siemens 10SG, were chosen for nitrite detection. The dipsticks were read by Roche Urisys 1100 and Siemens Clinitek Status+ reflectance photometers, respectively. First, a clean dipstick was dipped into a sample solution and wiped along the edge of the container to remove excess liquid. Then, the dipstick was placed onto the sample tray of the reflectance photometer and the on-screen instructions were followed. Typically, results will be generated by the instrument within 2 min of placing the dipstick on the sample tray.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c07205.

Table of performance attributes of nitrite detection assays and figures of electronic schematic of the colorimeter, sample volume versus measured nitrite concentration, detection of nitrite using four permutations of Griess reactions, characterization of Sigma nitrite detection kit and colorimeter, kinetics of the Griess reaction, transmitted intensity of RGBW LEDs versus nitrite concentration, calibration curve for nitrite detection in artificial urine, scattered intensity versus optical density for turbidity measurements, stability of detection reagents at room temperature, 4 °C, and −20 °C, and mobile application interface showing the measurement data (PDF)

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Notes

The authors declare no competing financial interest.

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