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ABSTRACT

This paper demonstrates that the gas-filled compartments in the packing material commonly called “bubble wrap” can be re-purposed in resource-limited regions as containers to store liquid samples, and to perform bioanalyses. The bubbles of bubble wrap are easily filled by injecting the samples into them using a syringe with a needle or a pipette tip, and then sealing the hole with nail hardener. The bubbles are transparent in the visible range of the spectrum, and can be used as “cuvettes” for absorbance and fluorescence measurements. The interiors of these bubbles are sterile and allow storage of samples without the need for expensive sterilization equipment. The bubbles are also permeable to gases, and can be used to culture and store microorganisms. By incorporating carbon electrodes, these bubbles can be used as electrochemical cells. This paper demonstrates the capabilities of the bubbles by culturing *E-coli*, growing *C. elegans*, measuring glucose and hemoglobin spectrophotometrically, and measuring ferrocyanide electrochemically, all within the bubbles.

Keywords: Adaptive use, bubble wrap, sterile, microorganism culture, electrochemical cell, low-cost cuvette.

INTRODUCTION

The storage, transportation, manipulation, and analysis of samples and reagents for applications in public health, agriculture, veterinary medicine, and environmental monitoring require appropriate containers. Samples and reagents are now mostly kept in rigid containers (e.g., sealed vials, microcentrifuge tubes, microtiter well plates, etc.).[1] These types of containers are convenient for use in well-funded laboratories, but they can be expensive, difficult to sterilize and dispose of, brittle (and thus capable of generating "sharps"), and sometimes may not be available or affordable in resource-limited settings. There could be a variety of uses for inexpensive, sterile containers for collection and storage of samples or reagents for analysis, either in the field, or in more centralized laboratories. In the case that these containers are transparent, they can also be used as cuvettes for performing colorimetric assays.

"Adaptive use" implies using materials already designed and produced in large quantity, with high quality at low cost, for purposes other than those for which they were originally intended. We and others have developed systems based on "adaptive use", for example, egg-beaters and CD player as centrifuges,[2] paper for microfluidic devices,[3,4,5,6] flatbed scanners and cell phone cameras as colorimetric detectors for paper-based microfluidic devices,[7] cell phones as microscopes,[8,9] phase change materials as incubators,[10] and bicycles as power generators.[11]

Flexible pouches made of polymeric films have found limited applications for reagents' storage. For example, Bau *et al*. used flexible pouches as reservoirs for pre-loaded liquid reagents or as pneumatic drivers in microfluidic devices.[12,13,14] A commercially available test kit for detecting bacteria by estimating the most-probable number of colony-forming units also uses a multi-well pattern of flexible pouches all connected to each other as suitable containers to split the sample in different wells.[15] Bubble wrap, which consists of flexible pouches partially filled

with air, can be filled with liquid samples. For example, Hart has prepared a form of art by injecting paint into bubbles of bubble wrap.[16] Bubble wrap has at least eight characteristics that make it attractive as a candidate for adaptive use as container for liquid samples. Bubble wrap is: i) readily available in almost all regions of the globe; ii) very inexpensive (~ $0.6/$m^2$; 1 m^2 provides 1000 to 5000 bubbles, depending on sizes); iii) lightweight; iv) available in a wide range of sizes; v) compartmentalized in a regular pattern (useful for parallel multi-bubble assays); vi) easily cut using scissors; vii) easily disposed of by burning; and viii) flexible (and unlikely to yield "sharps" when broken or torn). There are also obvious limitations to the adaptive use of bubble wrap as a container: i) the bubbles are only relatively stable to impact (especially punctures), and must be handled carefully; ii) the process of filling the bubbles with reagents requires the use of syringes; iii) the bubble wrap is sensitive to light due to the degradation of the film upon prolonged exposure to UV-rich light sources; and (iv) bubble wrap is bulky.

We found that the bubbles of bubble wrap are transparent, sterile and gas-permeable containers. Here, we demonstrate the potential for adaptive use of the bubbles of bubble wrap: a) as containers for storing liquid samples; b) as cuvettes for performing absorbance and fluorescence measurements; c) as containers for culturing bacteria and growing microorganisms; and d) as electrochemical cells, after inserting carbon electrodes in the bubbles. The bubbles of the bubble wrap, therefore, can be used for storing samples and performing analytical assays, a function that has the potential to be especially beneficial in resource-limited regions, and in very cost-sensitive applications.

EXPERIMENTAL SECTION

Materials and Chemicals. Bubble wrap was obtained from P&M Consolidator, Packing and Shipping Company. Acrylate-based nail hardener (New York Clear nail hardener 270A) and adhesive sealant (DAP® silicone) were obtained from local stores (Cambridge, MA). Syringes (3 mL), and needles (27 G ½), were obtained from Becton Dickinson. Hemoglobin, Drabkin's reagent, Brij 35, potassium ferrocyanide, Allura Red, rhodamine B, fluorescein, eosin Y, nitric acid, and copper were obtained from Sigma Aldrich. Carbon ink (E3456) was obtained from Ercon Inc. (Wareham, MA).

Absorbance and fluorescence measurements were performed using a microtiter plate reader (model SpectraMax M2, Molecular Devices). Chronoamperometric measurements were carried out using an electrochemical analyzer (model Autolab PGSTAT302N, Metrohm).

Storing Reagents in the Bubbles of Bubble Wrap. We injected solutions into bubbles of different dimensions using a syringe with a 27 G ½ gauge needle, and sealed the puncture with a clear nail hardener. The solutions can also be efficiently injected through a re-sealable adhesive patch, fabricated by casting a silicone adhesive onto a portion of the bubbles and curing it at 70 °C overnight. Alternatively, we used plastic pipette tips (0.1-10 μL, 20-200 μL, 100-1000 μL) to inject the solutions into the bubbles of the bubble wrap, and sealed the puncture with a clear nail hardener. To determine whether the compounds diffuse through the polymer of the bubbles, we performed a dye-penetration test.[17] We immersed sealed bubbles containing distilled water in aqueous solutions of different dyes e.g., rhodamine B, fluorescein, and eosin Y. After 1 hour, we rinsed the bubbles and checked whether dyes had penetrated to the inside of the bubbles by measuring the absorbance of the solutions within the bubbles. To test the capability of bubble wrap to contain different reagents (e.g., aqueous solutions, concentrated acids or bases, and non-

aqueous solvents), we stored concentrated sulfuric acid, 37 % w/v, concentrated ammonium hydroxide, 14.8 M, dimethyl sulfoxide (DMSO), acetone and hexane into the bubbles. We also tested the evaporation of aqueous solutions stored inside the bubbles by monitoring the mass of sealed bubbles filled with water as a function of time.

Using the Bubbles of Bubble Wrap as Cuvettes. To test the use of bubbles as cuvettes for absorbance and fluorescence measurements, we filled each bubble with different aqueous solutions of rhodamine B at concentrations ranging from 2.5 - 25 μM. The bubbles were completely filled with liquid and air in order to avoid the formation of wrinkles that would scatter light on the surface of the bubbles. To facilitate the measurement of absorbance and fluorescence using a plate reader, we placed each bubble separately on the cover of the microtiter plate. We compared the absorbance and fluorescence values from solutions stored in the bubbles to those stored in microtiter well plates.

Gas Permeability of the Bubbles of Bubble Wrap. We tested the permeability of the bubbles to H_2, He, NH_3, CO_2, CH_4, O_2, Ar, and NO_2 by checking the inflation of the bubbles upon exposure to the gas. With the exception of NO_2, we exposed the bubbles, contained in a tube sealed with septums on both ends; to an atmosphere of the respective gas using a syringe-needle connected to a gas cylinder, and attached another syringe-needle at the opposite end of the tube to maintain gas flow. For testing NO_2 gas, we placed the bubbles inside a closed tube that contained concentrated nitric acid and copper swarf. Upon reaction, these reagents form nitrogen dioxide.[18]

Growing Microorganisms within the Bubbles of Bubble Wrap. Initially, we tested the sterility of bubbles by filling them with 250 μL of autoclaved Luria-Bertani growth medium, and then incubating them at 37 ºC for four days. Using two different sizes of bubbles (one with a

diameter of 1 cm and one with a diameter of 2.5 cm); we tested seven bubbles of each type and took all the necessary precautions (e.g., using a new syringe in each injection) in order to limit the risk of cross contamination between experiments. The solutions did not become turbid; this observation suggested that microorganisms did not grow inside the bubble. We then inoculated 1 µL of *E. coli* (strain HB101) into fourteen bubbles, seven of each type, filled with autoclaved Luria-Bertani growth medium, and incubated them at 37 ºC. The turbidity of the medium indicated bacterial growth. We also grew *C. elegans* within the bubbles by filling each bubble with 250 µL of broth. The broth contained a culture of *E. coli* (strain HB101) in 2x yeast broth (16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl, adjusted pH 7.0); we then added 10 µL of a solution of broth containing six worms (at larval stage 4, when they are ~ 800 µm in length).[19] Using 1 cm diameter bubbles, we grew 42 *C. elegans* (six worms per bubble) for 12 days.[20]

The Bubbles of Bubble Wrap as Electrochemical Cells. We prepared sealed electrochemical cells using the bubbles and carbon electrodes. We prepared carbon electrodes by stripping the insulating layer from the tip of insulated copper wire, immersed this tip (~10 mm length) into carbon ink, and allowed the carbon coating to dry in air for 2 hrs. After inserting two carbon electrodes into each bubble, we sealed the puncture using clear nail hardener, and taped the rest of the electrodes to the surface of the bubbles. We filled the bubbles by injecting various concentrations of potassium ferrocyanide solutions (in 0.1 M aqueous KCl, used as model electroactive species), into them and sealed the puncture. We connected the electrodes to the electrochemical analyzer using crocodile clips, applied a constant potential (0.5 V), and recorded the chronoamperometric responses of different concentrations of potassium ferrocyanide solutions.

Use of the Bubbles of Bubble Wrap for Performing Bioanalyses. Using hemoglobin and glucose as examples, we demonstrated that the bubbles in bubble wrap can be used as containers to perform bioanalyses. For determination of hemoglobin, we used the cyanomethemoglobin method.[21] We filled the bubbles by injecting 2.4 mL of Drabkin's reagent (containing KCN, $K_3[Fe(CN)_6]$, K_2HPO_4 and Brij 35 detergent)[21] into them, and sealed them using clear nail hardener. We then injected 0.1 mL of different concentrations of hemoglobin solution (6–18 mM, the clinically relevant range), and sealed the bubbles using clear nail hardener. After mixing by rocking the bubbles, we incubated the solutions for 15 minutes, placed the bubbles on the cover of a well plate, and measured the absorbance at 540 nm using a plate reader. We prepared blank solutions that did not contain hemoglobin in the same way and subtracted their background absorbance from the absorbance of hemoglobin-containing solutions.

For the colorimetric detection of glucose in artificial urine, we pre-filled the bubbles with 250-μL of a solution of glucose oxidase-horseradish peroxidase (5:1) (75 units of glucose oxidase enzyme activity per mL of solution), and potassium iodide (0.6 M) in phosphate buffer (pH 6.4). We then injected separate bubbles with different concentrations of glucose in artificial urine[22] (0.5–18 mM). After 30 min, we recorded the results of the colorimetric assay by capturing the image with a digital camera (Nikon D50) or a scanner (Epson Perfection 1640 SU). A calibration curve was generated for different concentrations of glucose by digitizing the images in Adobe®Photoshop®, and measuring the reduction of the mean pixel intensities in RGB format (blue channel) of the sample against the blank sample.

RESULTS AND DISCUSSION

Storing Reagents in the Bubbles of Bubble Wrap. Using aqueous solutions of dyes (e.g., rhodamine B, Allura Red) for easy visualization, we demonstrated that solutions can be stored efficiently within bubbles of different sizes (Figure 1). We injected the samples directly into the bubbles and sealed the punctures using nail hardener or through re-sealable, self-sealing silicone adhesive (Figure 1). The puncture was sealed after allowing the nail hardener to dry for 5 minutes, or after withdrawing the needle used to inject the solution through the adhesive. Plastic pipette tips (e.g., for dispensing 0.1-10 μL, 20-200 μL, 100-1000 μL of solutions) can also be used for injecting the sample into the bubble wrap, however, the larger the diameter of the pipette tip, the more nail hardener is needed to seal the puncture (Figure S1).

To determine the efficiency of sealing the bubbles, we performed a dye-penetration test by immersing bubbles filled with water into aqueous solutions of various dyes, including rhodamine B (cationic), fluorescein (neutral), and eosin Y (anionic) for 1 hour. After rinsing the bubbles with water, we measured the absorbance of aqueous solutions within the bubbles. We did not observe any absorbance peaks for the dyes indicating that the dyes did not penetrate to the interior of the bubbles. This result suggests that samples can be effectively sealed inside the bubbles without the risk of contamination from compounds (at least compounds of molecular weights associated with molecules of intermediate size) present outside the bubble.

Bubbles of bubble wrap can be used to store different kinds of reagents (e.g., aqueous solutions, concentrated acids or bases, and non-aqueous solvents). For example, we verified that bubbles of bubble wrap can store concentrated sulfuric acid solution, concentrated ammonium hydroxide solution, and DMSO; however, hexane and acetone could not be stored in the bubbles because these solvents dissolved the nail hardener used for sealing the punctured bubbles.

Aqueous solutions stored in the bubbles sealed with nail hardener evaporated slowly (~7% loss in volume per week at 23 ˚C), determined *via* monitoring the mass of sealed bubbles filled with water. The mechanism of this loss is likely diffusion of vapor across the polyethylene film (a semi-permeable membrane). The bubbles can thus be conveniently used to store aqueous reagents for various analytical assays for several weeks and for longer periods in closed or humidified containers.

In resource-limited settings, biological samples such as blood and urine are often transferred as dried spots on paper. The assays that can be performed in dried blood or urine are, however, more limited than those that can be carried out using whole blood or urine. The low cost-per-bubble (< $0.0001) and the ability of bubble wrap to store different amounts of fluid samples, from few microliters to several milliliters (depending the size of the bubble), enable bubble wrap to be used in resource-limited settings to store fluids – originating from humans, animals, foods, or beverages – and transport them to centralized laboratories of analysis.

Using the Bubbles of Bubble Wrap as Cuvettes. The bubbles of bubble wrap are transparent containers that can be conveniently used to perform absorbance and fluorescence measurements in the visible range of the spectrum by using microtiter plate readers (Figure 2A). We prepared calibration plots of absorbance and fluorescence intensity against concentrations of rhodamine B ranging from 2.5 - 25 μM, using a plate reader. Absorbance and fluorescence emission intensity correlates linearly with concentration (in the range 2.5 - 25 μM) for the rhodamine B dye contained in both well plates and the bubbles of bubble wrap (Figure 2B and 2C). The linear fits of the experimental data have slopes that are linear and similar, but not exactly the same. This difference could be attributed to the fact that optical path lengths are slightly different, but regardless of its origin, this correlation confirms that the material of the

bubble wrap does not interfere with the measurement, and that bubble wrap can be used as a cuvette for analytical absorbance and fluorescence measurements. To enable routine analysis with bubble wrap using a microtiter plate reader, the plate reader would only need to be programmed to measure absorbance and emission from the hexagonal arrays of bubbles typical of bubble wrap.

Gas Permeability of the Bubbles of Bubble Wrap. We explored the permeability of the bubbles of bubble wrap to various gases, namely H_2, He, NH_3, CO_2, CH_4, O_2, Ar, and NO_2. In all cases, after exposure of partially inflated bubbles to an atmosphere of a gas, the bubbles inflated completely; this observation suggests that the bubbles are gas-permeable. The transport of gas molecules through a polymer membrane can occur by dissolution in the membrane and diffusion, or by permeation through micro- or nano-pores; transport would be driven by the entropy of dilution of the gas in the air in the partially inflated bubble.[23] Under steady-state conditions, to a first approximation, diffusion obeys Fick's law. If the diffusion coefficient is independent of gas concentration, and the solubility of the gas in the membrane obeys Henry's law, then Fick's first law can be integrated to yield the approximate relationship: $P = DS_0$, where P is the permeability coefficient ($cm^3 \times cm / cm^2 \times s \times Pa$), D the diffusion coefficient (cm^2/s) and S_0 the solubility coefficient ($cm^3(STP)/cm^3 \times Pa$) in Henry's law.[24] There are many problems, however, involved in an attempt to compare and interpret experimental data on models for the diffusion in polymers.[25] Another experimental way to estimate the permeability coefficient is by using the following equation: $P=VL\times10^{10}/(At\Delta p)$, where P is the permeability in Barrer (1 Barrer = 10^{-10} cm^3 (STP)$\cdot$ cm /$cm^2 \cdot$ s $\cdot$ cmHg), V the permeated gas volume in cm^3, L the membrane thickness in cm; A the effective area of the membrane in cm^2, t the measurement time in seconds, and Δp the pressure difference between the two sides in cmHg.[26] A quantitative ranking

of the rate of inflation in decreasing order in our experiments was as follows: H_2 > NH_3> He > CO_2 > CH_4 > O_2 > Ar. The trend in rate of inflation do not seem to correlate with molecular weight or molecular volume of the gases, or with their previously reported permeability coefficients (P) across low-density polyethylene (e.g., P, in Barrers, reported to be: He = 4.9; CH_4 = 2.9; O_2 = 2.9; and CO_2 = 12.7).[27] Figure S2 shows examples of swollen bubbles after exposure to ammonia and nitrogen dioxide. In the case of exposure to nitrogen dioxide, we observed a brown color inside the bubbles. To confirm that the brown gas was inside the bubbles and not adsorbed on the surface, we withdrew a sample from the bubbles using a syringe. The bubbles deflated, and turned colorless (Figure S2B). For the bubbles exposed to ammonia gas, we rinsed the inflated bubbles with water to remove any residual ammonia that might be adsorbed on the surface of the bubbles and then injected a phenolphthalein-containing solution into the bubbles. As expected, we observed a purple color inside the bubbles injected with phenolphthalein; this color is consistent with the reaction of the indicator with $NH_4^+OH^-$. The bubbles without injected phenolphthalein remained colorless (Figure S2C).

The bubbles that were punctured and then sealed with nail hardener also inflated, and remained swollen for approximately 30 minutes after removal from the chamber, suggesting that the nail hardener efficiently sealed the bubbles. The gas permeability of bubble wrap — and especially its permeability to oxygen — allow the use of bubbles as containers for culturing and storing microorganisms.

The Bubbles of Bubble Wrap are Sterile Containers. After filling the bubbles with autoclaved growth medium and incubating them at 37 °C for four days, we did not observe turbidity of the medium; this observation suggests that there was no microbial growth in the bubbles (columns 3 and 5, Figure 3A). Oxygen diffusion was not a limiting factor for the growth

of bacteria in the bubbles because we proved that bubbles are permeable to oxygen. The absence of turbidity thus suggested that the interior of the bubbles is sterile.

Bubble wrap is usually made from polyethylene film. The film is wrapped onto a cylinder with holes of pre-defined diameter and a vacuum is applied to form the bubbles in which air is trapped when the film is thermally laminated with a second layer. We believe that the high temperatures (~80−100°C) used in the fabrication of bubble wrap could render the interior of the bubbles sterile.

Growing Microorganisms within the Bubbles of Bubble Wrap. After inoculating some of the bubbles filled with autoclaved growth medium with 1 µL of solutions containing *E. coli* (strain HB101), and then incubating the bubbles at 37 °C, the medium within the bubbles became turbid after 16 h, and increasingly cloudy over 4 days (column 1, Figure 3A). The bacteria thus multiplied inside the bubbles. Using bubbles with a diameter of 1cm, we also grew 42 *C. elegans* (six worms per bubble) for 12 days. The broth solution within the bubbles contained *E. coli* and yeast to feed the worms. The *C. elegans* contained in the bubbles grew to ~ 1 mm length as illustrated (for a single bubble) in Figure S3. *C. elegans*, therefore, lived, grew, and multiplied in the bubbles (containing broth of *E. coli* culture and yeast). This result shows that even multicellular organisms can survive and multiply within the bubbles of bubble wrap.

Bubble wrap can therefore be used as a storage container for microorganisms: it is sterile and permeable to air. Storing microorganisms in a container that allows them to stay alive while being transported is an important capability, for example, when analyzing bacterial contaminations of water supplies.

The Bubbles of Bubble Wrap as Electrochemical Cells. By inserting two carbon electrodes into each bubble of the bubble wrap that was filled with a potassium ferrocyanide

solution, we demonstrated, using chronoamperometry, that the bubbles can be used as electrochemical cells. We recorded the chronoamperometric response of different concentrations of potassium ferrocyanide solution (in 0.1 M KCl), while applying a constant potential of 0.5 V (Figure 4). We calculated the average steady-state Faradaic currents measured between 50-60 s after applying the potential. A calibration plot of Faradaic current against the concentration of ferrocyanide ion is linear (Figure 4), consistent with the Cottrell equation.[28]

Use of the Bubbles of Bubble Wrap for Performing Bioanalyses. The bubbles of bubble wrap can be used as containers for running bioassays; the "wells" are preformed, transparent, and organized in a regular pattern. By filling the bubbles with reagents for bioassays and sealing the punctures (or by injecting reagents through a re-sealable adhesive), the bubble wrap can be carried into the field for running assays on-site. As an example of potential application, we demonstrated the colorimetric detection of hemoglobin and glucose by using bubbles pre-filled with the necessary reagents. All the experiments were carried out in laboratory settings but the same experimental procedures can easily be performed in the field.

The hemoglobin concentration in blood is an important biomarker for detection of anemia (< 14 g/dL in men, and < 12 g/dL for women).The cyanomethemoglobin method is the gold standard method for the determination of hemoglobin.[29,30,31] In this method, the ferricyanide in Drabkin's reagent oxidizes the iron in hemoglobin, converting hemoglobin to methemoglobin. The cyanide, contained in Drabkin's reagent, reacts with methemoglobin to form cyanomethemoglobin, and generates a color that can be measured spectrophotometrically (λ_{abs} = 540 nm).[32,33,34] The main drawback of this method is that it requires the handling of reagents and solutions that contain cyanide, a highly toxic compound. The wastes that are produced from the cyanomethemoglobin method should be disposed of appropriately.[35,36]

Bubble wrap is a suitable container for the point-of-care determination of hemoglobin using the cyanomethemoglobin method. The Drabkin's reagent could be stored inside the bubbles in central facilities, and the filled bubbles can be safely transported to the field as they are sealed. The end user would only need to inject the blood sample into the bubble and seal the puncture with nail hardener; the sample would then react with the Drabkin's reagent to generate concentration-dependent intensities of the characteristic brown color. The results could be read immediately by comparing the color with a panel of standards, or the used bubbles can be collected, transported back to the central facility and be measured with a plate reader. In both cases after the measurement, the reagents stored in the bubbles should be disposed of appropriately. The fact that the wastes are sealed inside the bubbles facilitates their disposal. The collection of liquid wastes generally involves accumulation in closed containers, which may contain other wastes. The wastes that are sealed inside the bubble wrap can easily be transferred to the appropriate facilities with decreased risk of spilling or mixing with other incompatible wastes. Small quantities of fluids can also be incinerated directly in bubble wrap but not in glass or plastic containers.

After adding different concentrations of hemoglobin to Drabkin's reagent pre-stored in the bubbles, the color changed from light yellow to dark brown. The intensity of the absorbance (at 540 nm) of the solutions in the bubbles correlated linearly with hemoglobin concentration in the clinically relevant range (6 - 18 g/dL); we observed similar correlations for the hemoglobin contained in both the wells of 96-microtiter plates, and the bubbles of bubble wrap (Figure 5).

For the determination of glucose in artificial urine, we pre-stored all the necessary reagents, enzymes, colorimetric reagents, and buffer solutions, in the bubble; the end user thus has only to inject the sample. We prepared the calibration line of the reduction of the mean pixel

intensities in RGB format (blue-channel) *vs* the concentration of glucose injected in the bubble in the range of 0.5–18 mM (Figure 6) and we noticed that the reduction of the value of the blue channel correlated linearly with the concentration of glucose.

We tested the performance of the assay with reagents stored in the bubbles over a period of two weeks by running assays at two-day intervals using a 12-mM standard of glucose. The mean pixel intensity (in RGB format, blue-channel) on day 1 was statistically indistinguishable (from a t-test) to the intensity measured on day 14. We thus verified that the reagents remain active and stable in the bubbles for 14 days at 23 ºC, with no observable loss in accuracy for detecting glucose.

We also checked the robustness of the assay to different lighting conditions by measuring two samples containing 6.5 and 13.5 mM glucose and obtained values of 5.8±1.6 mM and 11.9±0.6 mM, respectively, based on calculations from the calibration line; these experimental values represent the averages and standard deviations of seven independent measurements.

CONCLUSIONS

Bubble wrap has five properties that allow it to be adaptively used as a container for storing liquid samples and performing analytical assays. The bubbles are: i) easily filled using a syringe with needle or a pipette with a plastic tip and sealed using nail hardener; ii) sterile; iii) gas permeable; iv) chemically inert to most aqueous samples; and v) optically transparent in the visible region of the light spectrum, (useful for visual examination and optical measurement).

Sterile containers for storing reagents and biological samples for bioanalyses are not readily available in resource-limited settings, and sterilization equipment (e.g., autoclaves) are expensive. Bubble wrap can be re-purposed and re-used as a sterile container for storing liquid

samples without the need for sterilization. This property may be useful for simple bioanalyses in resource-limited regions. For example, the sterile bubbles can be used to perform microbial culture tests.

The bubbles of the bubble wrap are transparent and can be used as containers for absorbance and fluorescence measurements. Reagents for spectrophotometric measurement of hemoglobin in blood or glucose in urine, for example, can be stored within the bubbles and transported within sealed bubbles. The sealed bubbles prevent the contact of users with the reagents and eliminate contamination of the reagents or exposure of end users to toxic compounds, such as those used for the cyanomethemoglobin method.

The possibility of using the bubbles as electrochemical cells extends the scope of analytical assays that can be performed using bubble wrap. Electrochemical analysis is insensitive to light, dust and impurities that can be a problem in optical measurements.

Bubble wrap is readily available in almost all regions of the globe. The adaptive use of bubbles of bubble wrap for storing liquid samples and performing analytical assays can reduce the cost of analysis, by significantly reducing the cost of the necessary storage containers. The cost of the containers is only a fraction of the cost of the overall procedures which also includes the cost of the necessary reagents, the instrumentation and the trained personnel; however, in resource-limited settings, any reduction of the cost of analysis is important towards making the test procedures affordable for the people.

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SUPPORTING INFORMATION AVAILABLE

Figures S1-S3. This information is available free of charge via the Internet at http://pubs.acs.org.

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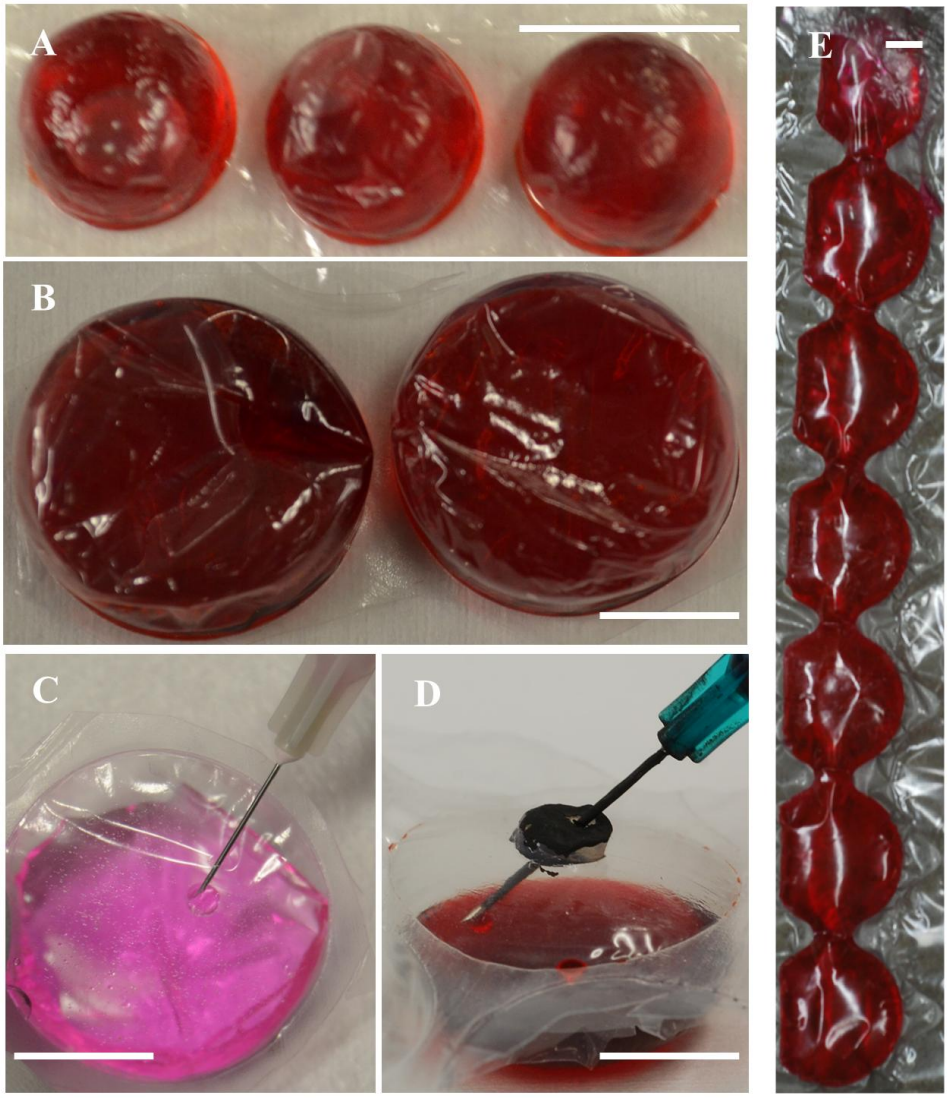

Figure 1. (A) Bubbles with a diameter of 1 cm filled with 300 µL of an aqueous solution Allura Red dye. (B) Bubbles with a diameter of 2.5 cm filled with 5 mL of Allura Red dye. (C) Filling a bubble with a diameter 2.5 cm by injecting an aqueous solution of rhodamine B using a needle and syringe. (D) Filling a bubble with a diameter 2.5 cm by injecting an aqueous solution of rhodamine B using a needle and syringe through a re-sealable silicon adhesive. (E) Continuous compartments of bubbles filled with 30 mL of an aqueous solution of Allura Red dye. All scale bars represent 1 cm.

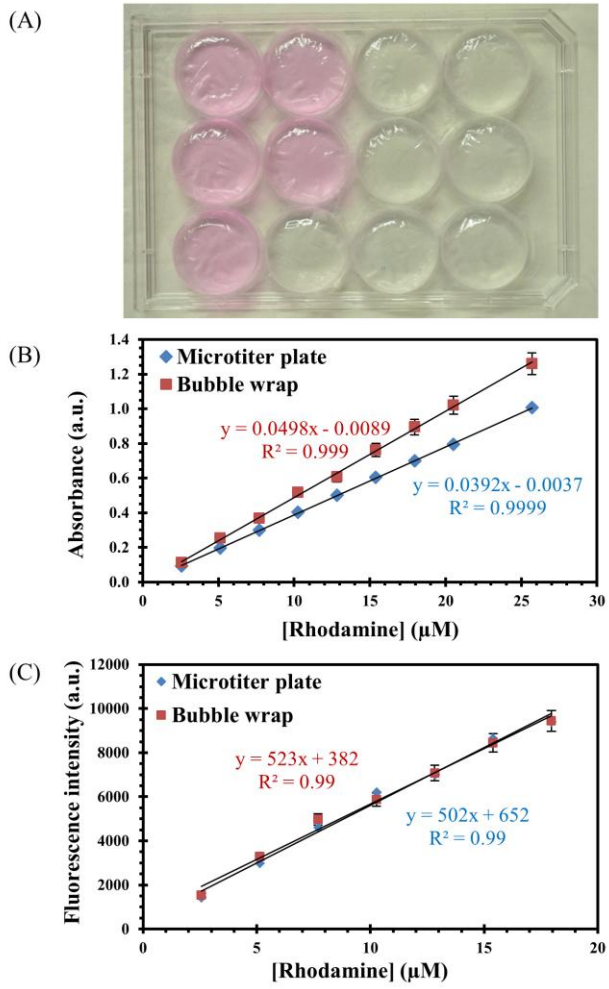

Figure 2. (A) Bubbles filled with an aqueous solution of rhodamine B placed on a cover of a microtiter plate to be measured by the microplate reader. (B) A calibration plot of absorbance (at λ_{max} = 550 nm) vs concentration (2.5 – 25 μM) for rhodamine B obtained for closely matched path length of the dye in the microtiter plates and in the bubbles. The data were fit by linear least-squares regression. Each datum is the mean of seven replicate absorbance measurements and the error bars represent the standard deviations of these measurements from the mean value. (C) A calibration plot of fluorescence emission (λ_{exc}/ λ_{em} = 490/580 nm) vs concentration (2.5 – 25 μM) of rhodamine B measured in microtiter wells and in the bubbles of bubble wrap.

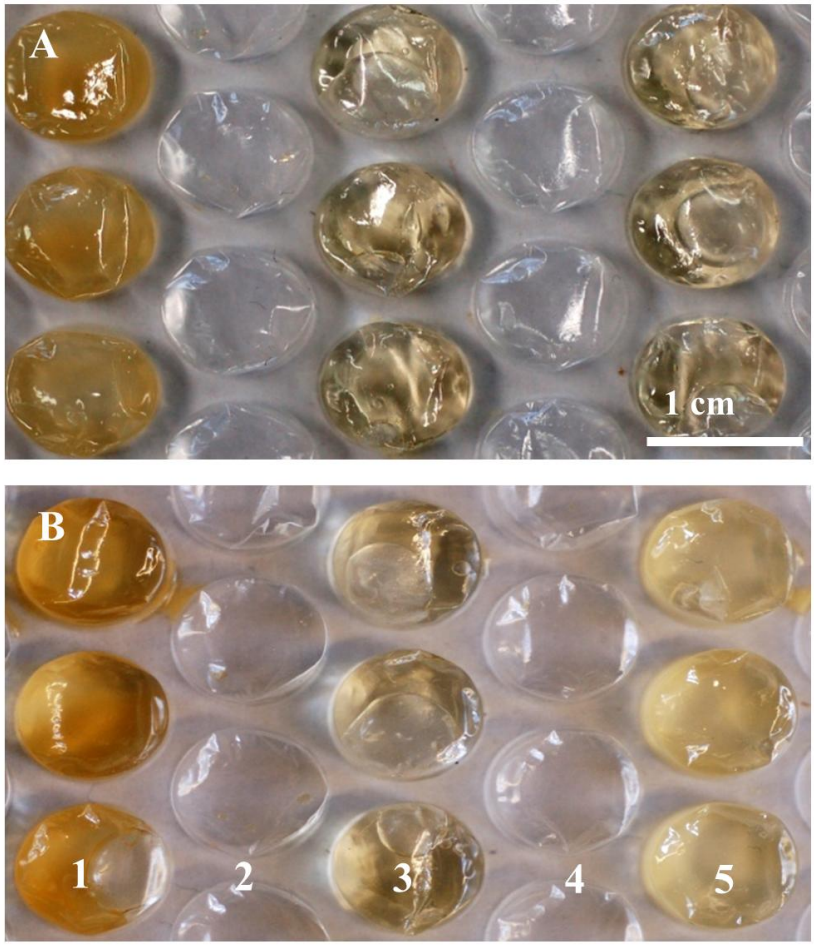

Figure 3. (A) The bubbles in columns 1, 3, and 5 were filled with 250 µL of yeast extract and tryptone growth medium; bubbles in columns 2 and 4 were not used. The bubbles in column 1 additionally contained 1 µL of *E. coli* (in growth medium). All bubbles were incubated at 37 ºC for four days. The bubbles became more turbid in column 1, due to growth of *E. coli*, compared to bubbles in columns 3 and 5 in which no bacteria was added, suggesting that the bubbles are sterile. (B) Images of the bubbles after transferring a 1 µL sample of *E. coli* from column 1 to column 5. The medium became turbid in the bubbles in column 5 after incubating the bubble wrap for 16 h at 37 ºC, due to the growth of *E. coli*.

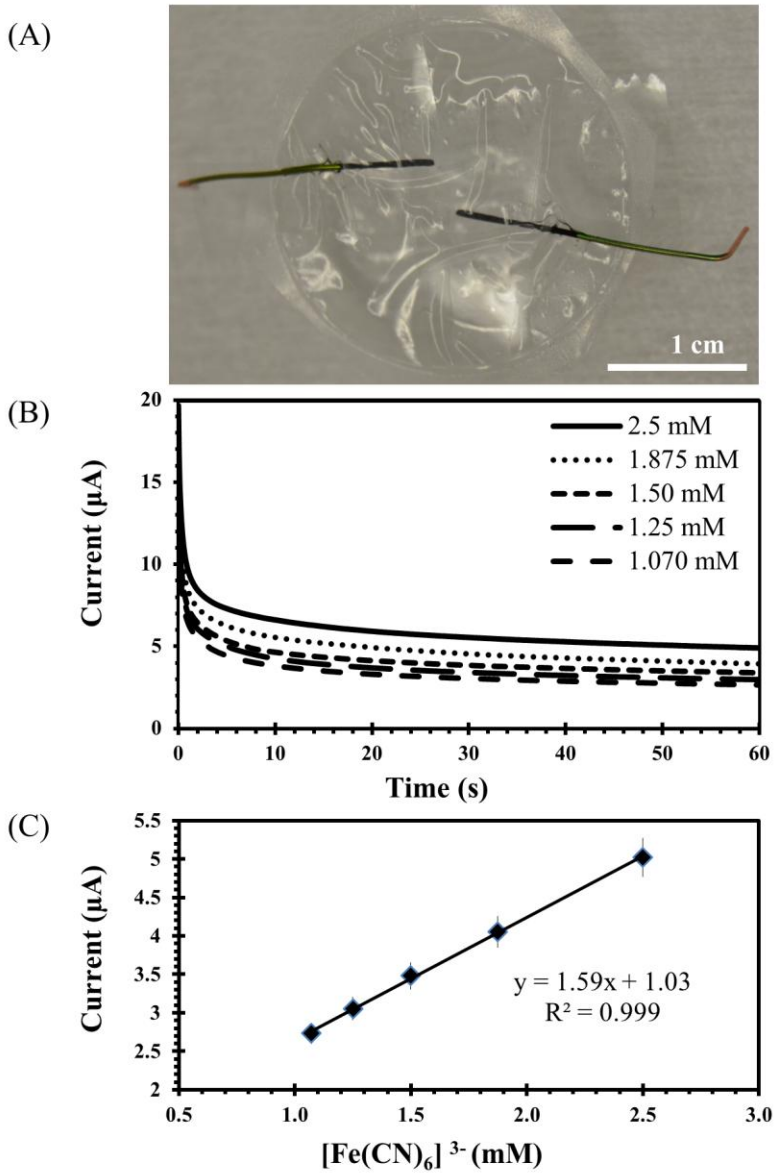

Figure 4. (A) Sealed electrochemical cell made of bubbles and carbon electrodes before electrochemical measurements. The bubbles were filled with the solutions and sealed with nail hardener. (B) Chronoamperometric responses of different concentrations of ferrocyanide, and (C) the calibration plot of steady-state current against different concentrations of ferrocyanide at an applied potential of 0.5 V. The error bars represent the standard deviation from the mean of seven measurements.

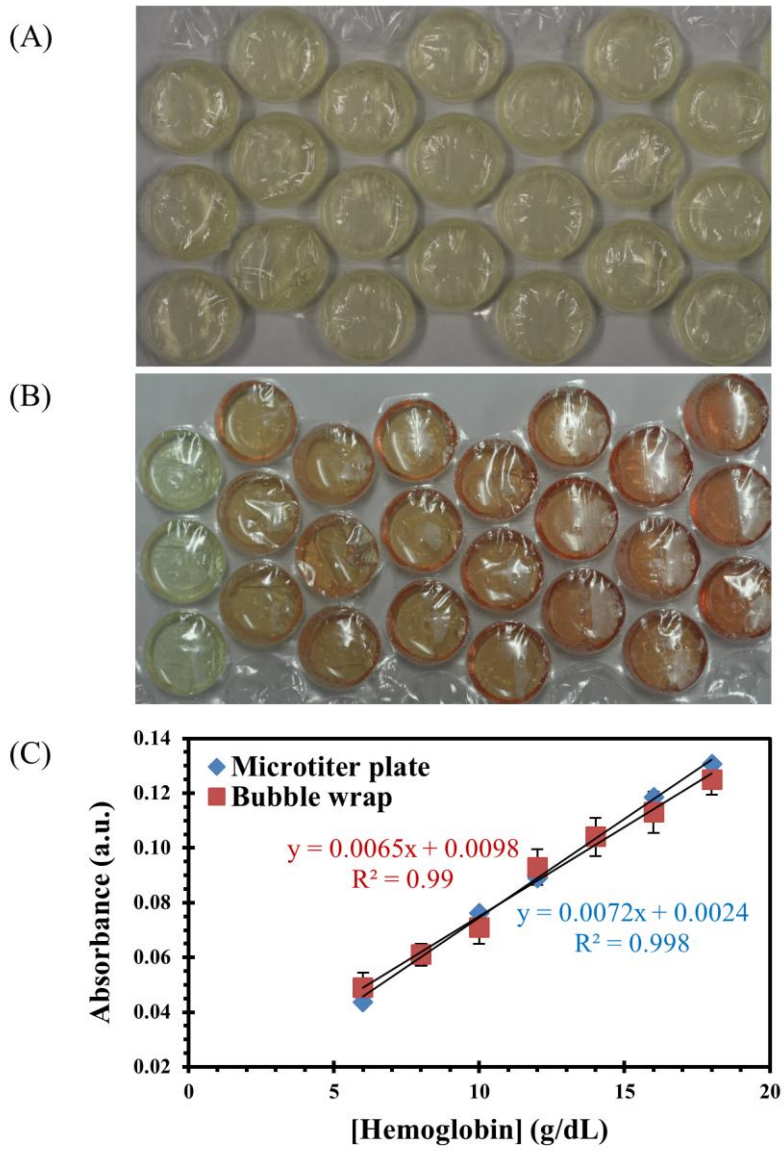

Figure 5. (A) Bubbles filled with Drabkin's reagent, and (B) bubbles filled with Drabkin's reagent and varying concentrations of hemoglobin. We measured the absorbance of the solutions using a plate reader. (C) Calibration plot of absorbance (at λ_{max} = 540 nm) vs concentration of hemoglobin obtained for approximately matched path length of the dye in the well plates and in the bubble wrap. The data were fitted by linear least-squares regression. Each datum is the mean of seven replicate absorbance measurements and the error bars represent the standard deviations of these measurements from the mean value.

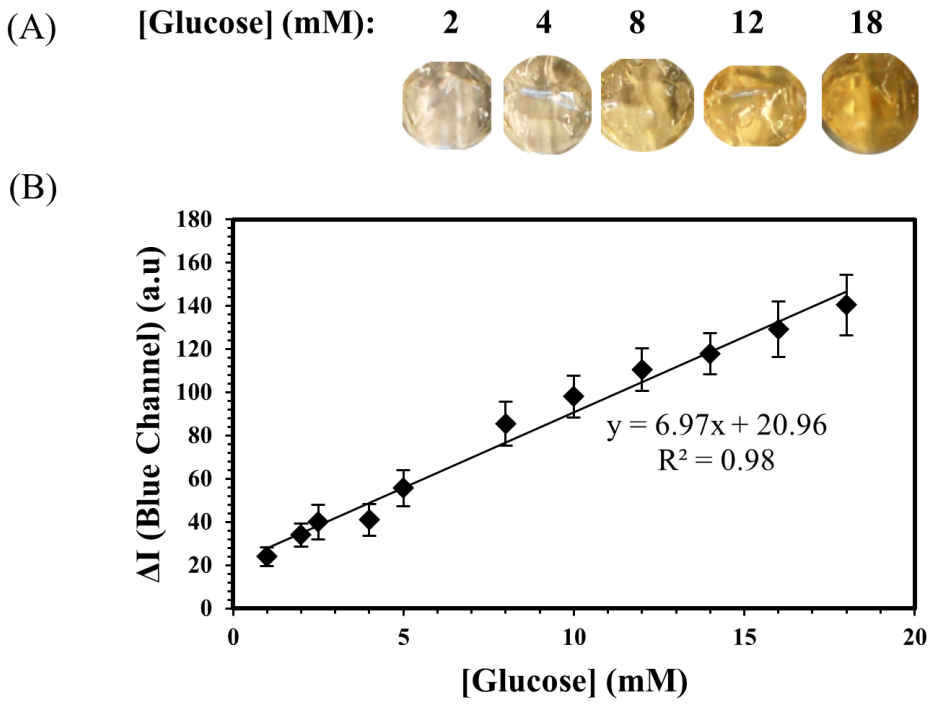

Figure 6. (A) Pictures of bubbles containing different concentrations of glucose in artificial urine samples. (B) Calibration line of the reduction of the intensity of the blue channel vs the concentration of glucose (0.5–18 mM) in artificial urine using bubbles as containers. Each datum is the mean of a minimum of seven assays and the error bars represent the standard deviations of these measurements from the mean value.

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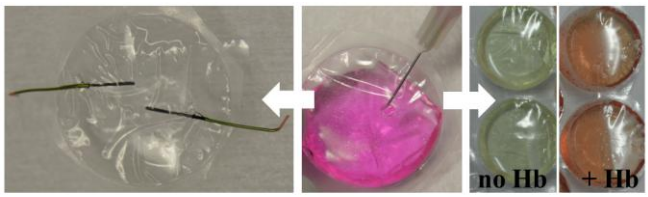

Electrochemical cell **Inject & Seal** Cuvette